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CONTENTS

PAGE

Production of Immunity against *Clostridium chauvoei* Infection in Sheep. *M. B. Buddle* 395

Labour-saving Devices in Soil Testing. *D. E. Hogg* and *D. F. Waters* 414

Effect of Pregnant Mare Serum on the Reproductive Performance of Romney ewes. *L. R. Wallace, L. J. Lambourne* and *D. P. Sinclair* 421

Experiments in Control of Peach Brown-rot. *P. J. Brook* 437

Blast of Stone-fruit in New Zealand. *D. W. Dye* 451

Differential Effect of Therapeutants on Bacterial Canker of Tomatoes and on Late Blight. *D. W. Dye* 462

Grass-grub Populations in Soils of the Manawatu Sand Country. *R. A. Cumber* and *J. D. Cowie* 465

NOTICES

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PRODUCTION OF IMMUNITY AGAINST *Clostridium chauvoei* INFECTION IN SHEEP

By M. B. BUDDLE, Animal Research Station, Department of Agriculture, Wallaceville

(Received for publication, 11 September 1953)

Summary

1. Vaccination against blackleg in New Zealand was initiated in 1939 subsequent to the confirmation of *Clostridium chauvoei* infection as responsible for sheep mortalities in 1938.

2. Following preliminary field trials, blackleg vaccine as prepared at Wallaceville for use in cattle was issued free for the control of *Cl. chauvoei* infection in sheep.

3. Immunity trials in sheep in 1948 indicated that the Wallaceville blackleg vaccine and an Australian commercial product were of low potency.

4. Alum precipitation of the Wallaceville vaccine significantly enhanced the efficiency of the product.

5. Experimental vaccines prepared in beef-infusion glucose-cysteine medium and alum precipitated induced a high degree of immunity in sheep.

6. A technique suitable for the large scale production of a potent blackleg vaccine was developed.

7. The procedure developed for potency testing of blackleg vaccines is described.

8. Immunity trials were conducted with sheep to assess the efficiency of experimental and commercial blackleg vaccines.

9. The problem of production and persistence of immunity in hoggets using the improved type of vaccine was studied.

10. Recommendations for the most effective use of the vaccine for the control of *Cl. chauvoei* infection in sheep under field conditions in New Zealand are suggested.

11. In 1949 approximately 400 000 doses of the improved type of blackleg vaccine were issued from Wallaceville for the immunization of sheep.

12. All commercial blackleg vaccine available since March 1950 for use in New Zealand has been the improved product, prepared by the Wallaceville technique and subjected periodically to tests for potency in sheep.

INTRODUCTION

Blackleg in cattle was first recognised in New Zealand in 1891, and vaccination of calves for the control of the disease was initiated in 1903. By 1906, over 33,000 calves were vaccinated annually with a heat-treated infected muscle tissue vaccine prepared at Wallaceville. This type of vaccine was used exclusively for the immunization of cattle against blackleg in New Zealand until 1930. In this year, the original powdered product was replaced by a formalinized culture vaccine, the method of preparation being a modification of Green's technique (1929). As a result of immunity tests conducted on sheep and guinea pigs (Gill, 1931), a dose of 1 ml. of the new vaccine was recommended and subsequently used for the immunization of cattle.

Prior to 1938, *Clostridium chauvoei* infection was not recognized as contributing to sheep mortalities in New Zealand. Following the observations in this year, that *Cl. Chauvoei* infection was responsible for heavy mortalities in hoggets and other classes of sheep, an endeavour was made to determine the role of this organism in gas gangrene infections of sheep. Evidence rapidly accumulated over the next few years which indicated that *Cl. chauvoei* was predominantly responsible for gas gangrene infections in sheep consequent to such operations as lambing, shearing, dipping, lamb docking, marking and castration, experimental blood sampling and vaccination. *Cl. chauvoei* infection was also shown to be responsible for mortalities in new born lambs from an umbilical infection as well as for heavy mortalities in hoggets on root crops or less regularly on pasture, unassociated with any obvious trauma. Field vaccination trials were first undertaken in 1939 in an effort to control losses from *Cl. chauvoei* infection in sheep. The success of the original trials with hoggets resulted in extension of vaccination in endeavours to reduce the mortalities from *Cl. chauvoei* infection in hoggets and other classes of sheep. A number of variations in vaccination procedure was studied under field conditions, including such aspects as dosage, number of inoculations necessary utilising *Cl. chauvoei* or combined *Cl. chauvoei* and *Cl. septicum* vaccines. Finally, a single does of 2 ml. of the blackleg vaccine, as then prepared at Wallaceville for the immunization of calves, was adopted for the control of *Cl. chauvoei* infection in sheep on properties where existence of infection had been confirmed by bacteriological procedures. Blackleg vaccine for the immunization of sheep and cattle was issued free from Wallaceville until March 1950.

Reports from the field on the efficiency of the single dose of 2 ml. of the Wallaceville blackleg vaccine in controlling sheep mortalities from *Cl. chauvoei* in general, were satisfactory. However, in 1947, field evidence indicated that in heavily infected areas, adequate protection against natural infection was not induced regularly in immunized sheep, particularly in two-tooth ewes and hoggets. Consequently studies were undertaken to determine experimentally the degree of immunity induced in sheep using blackleg vaccines currently available in New Zealand and in other countries. Endeavours were then made to develop a more effective immunizing agent for the control of the ovine infection and, in addition, a convenient practical procedure for assessing experimentally in sheep, the relative efficiency of these biological products. The purpose of this report is to record some of

the experimental observations, the technique finally adopted for the production of an improved type of blackleg vaccine and the application of this improved vaccine to the control of *Cl. chauvoei* infections of sheep in New Zealand.

Preliminary Experimental Observations

A preliminary experiment was undertaken at Wallaceville in 1948, to obtain more precise information on the degree of immunity in sheep afforded by varying doses of the standard Wallaceville vaccine. For purposes of comparison, a proprietary blackleg vaccine manufactured and procured through the regular commercial channels in Australia was tested simultaneously in sheep. The sample of Wallaceville vaccine used in this test was selected at random from supplies available for field use.

TABLE I. EXPERIMENTAL EVALUATION OF EFFICIENCY OF NEW ZEALAND AND AUSTRALIAN VACCINES (AVAILABLE IN 1948) IN PRODUCING IMMUNITY IN ROMNEY EWES AGAINST *Cl. chauvoei.*

(Animals challenged 3-4 weeks after vaccination)

Treatment	Dose of Virulent Culture (ml.)	Number Challenged	Number of Survivors
2 ml. old type New Zealand blackleg vaccine	0.02	1	0
	0.05	3	1
	0.1	1	0
	0.25	2	0
	0.5	2	1
	1.0	1	0
5 ml. old type New Zealand blackleg vaccine	0.05	2	2
	0.1	6	4
	0.25	3	0
	0.5	2	0
	1.0	1	0
5 ml. Australian Commercial blackleg vaccine	0.05	2	2
	0.1	5	3
	0.25	5	0
	0.5	5	1
	1.0	2	0
5 ml. old type New Zealand blackleg vaccine ALUM PRECIPITATED	0.25	1	1
	0.5	1	1
	1.0	1	1
	2.0	5	2
Controls	0.001	2	1
	0.002	2	0
	0.005	4	1
	0.01	4	0
	0.05	5	0

The preliminary tests, which are presented in summarised form in Table I, involved observations on 68 full-mouth non-pregnant Romney ewes. The experimental animals had not been vaccinated previously against blackleg and were procured from an area where the naturally occurring disease was unknown. Ten animals were inoculated subcutaneously with 2 ml. of the Wallaceville blackleg vaccine, 14 with 5 ml. of the same vaccine, and 19 with 5 ml. of the Australian proprietary blackleg vaccine (the recommended dose for sheep and cattle).

A quantity of the Wallaceville vaccine was precipitated by the addition of a 20 per cent sterile solution of potash alum at room temperature to make a final concentration of 2 per cent alum. Eight ewes were inoculated subcutaneously with 5-ml. doses of this product.

A group of seventeen ewes were left unvaccinated to assess the virulence of the broth culture of *Cl. chauvoei* used for the experimental inoculation of the vaccinated animals.

The vaccinated ewes were artificially infected, after an interval of between three to four weeks following immunization, by the intramuscular inoculation into the medial aspect of the thigh of a broth culture of a recently isolated strain of *Cl. chauvoei*. The following procedure was adopted for the artificial infection of vaccinated and control sheep in this and in later experiments. The bone marrow from a rib of a sheep that had died from the naturally occurring disease, was subcultured on the surface of a blood-agar plate. After 24 or 48 hr. incubation in an anaerobic jar, isolated colonies of *Cl. chauvoei* were inoculated into tubes of sheep-heart medium, prepared by adding approximately one gram of fresh finely minced fat-free sheep heart to 10 ml. of beef infusion broth in tubes, and sterilising by autoclaving. Prior to inoculation, the tubes were boiled for ten minutes and rapidly cooled. After inoculation the tubes were incubated in an anaerobic jar for 18 to 24 hr. One millilitre of the culture was inoculated intramuscularly into a guinea pig, and on death of the animal, the heart blood was removed aseptically and inoculated into freshly boiled and cooled sheep-heart medium. The tubes of inoculated sheep-heart medium were incubated in an anaerobic jar for 18 to 24 hr. Immediately prior to the inoculation of sheep the contents of several tubes were pooled. When the culture is required to be diluted, dilutions of culture are prepared in recently boiled and cooled broth, immediately prior to the inoculation of sheep. Sheep inoculated with these cultures usually died from 24 to 48 hr. after exposure, although deaths did occur from 18 to 96 hr. after inoculation, depending on the size of the inoculum and the degree of immunity of the experimental animal.

In the first trial, control sheep failed to survive the intramuscular inoculation of a dose of 0.001 ml. of culture of virulent *Cl. chauvoei*. Of seventeen unvaccinated sheep exposed to artificial infection only two survived and these two had received doses of 0.001 ml. and 0.005 ml. of culture. This test furnished confirmatory evidence of the high degree of susceptibility of sheep to *Cl. chauvoei* infection. The sheep vaccinated with 2.0 ml. of the N.Z. blackleg vaccine showed a low degree of immunity when exposed to virulent infection three to four weeks later.

In the preliminary trial, it appeared that the antigenicity of the New Zealand and Australian vaccines was of a similar degree. However, the degree of immunity induced by these vaccines in the recommended doses, viz. 2 ml. for the New Zealand product and 5 ml. for the Australian vaccine, would not appear to be of a very high order, even at the period of maximal response.

The addition of potash alum solution to the New Zealand blackleg vaccine resulted in a product of enhanced efficiency.

Two sheep of five immunized with 5 ml. of the alum-precipitated New Zealand vaccine survived the artificial exposure of 2 ml. of virulent culture. Three sheep which had been immunized with 5 ml. of the same vaccine which had not been treated subsequently with alum, failed to survive artificial infection with 0.25 ml. of virulent culture.

EXPERIMENTAL PROCEDURE

Trials with Vaccines prepared in a tissue-free medium

Rajagopalan (1947) had reported that a potent blackleg vaccine could be prepared in a beef-infusion medium containing glucose and cysteine HCl. No data were available to indicate the relative efficiency of vaccine prepared in this manner with vaccines prepared by more conventional procedures. A vaccine was prepared following the technique recommended by Rajagopalan. A quantity of this vaccine was alum precipitated by the addition at room temperature of sterile 20 per cent potash alum solution to make a final concentration of 2 per cent alum. The efficiency of these two vaccines was tested by the inoculation of sheep with 5 ml. doses of the two products, and after an interval of four weeks the immunized animals and unvaccinated sheep were inoculated intromuscularly with a culture of virulent *Cl. chauvoei* organisms.

The following technique was adopted for the preparation of the experimental vaccines:—

Basal Medium: To every 500 g. of finely minced fat-free beef, one litre of tap water was added. This was mixed and allowed to stand in the cold room overnight. The meat infusion was raised to boiling point and allowed to simmer for 30 min. The meat particles were removed by straining through muslin. When cold, tap water was added to adjust the broth to the original volume, and 0.5 per cent sodium chloride and 1 per cent peptone were added. The pH was adjusted to 7.0 and the broth was heated at 100° c. for 30 min. The broth was then filtered through moistened Chardin paper. Glucose, 0.5 per cent, and cysteine HCl, 0.05 per cent, were added and the pH was adjusted to 6.8. The broth was distributed in five-litre quantities in Pyrex flasks and the medium was sterilised by autoclaving at 15 lb. pressure for 20 min. After autoclaving the medium was cooled rapidly to 40° c. The medium was inoculated with a 2 per cent volume of an 18-hr. culture of *Cl. chauvoei* in the beef infusion glucose cysteine medium. Incubation of the inoculated medium was continued for 48 hr. at 37° c. Formalin was then added to a concentration of 0.5 per cent, sterile rubber bungs replaced the cotton wool

plugs and incubation was continued for a further 24 hr. After bacteriological and biological tests had confirmed the sterility of the vaccine, potash alum solution was added to a quantity of the product to make a final concentration of 2 per cent alum.

The results of the sheep immunity tests with the two types of experimental vaccines are presented in Table II.

TABLE II. PRODUCTION OF IMMUNITY IN ROMNEY EWES WITH EXPERIMENTAL VACCINES PREPARED IN TISSUE-FREE BROTH

(Animals challenged 4 weeks after vaccination)

Treatment	Dose of Virulent Culture (ml.)	Number Challenged	Number of Survivors
5 ml. Formalinized broth culture	0.25	2	2
	0.5	4	1
5 ml. Formalinized broth culture	10.0	1	1
	11.0	2	2
ALUM PRECIPITATED	12.0	3	2
Controls	0.0001	2	1
	0.0005	2	0
	0.001	2	0

In this experiment unvaccinated ewes failed to survive the intramuscular inoculation of 0.001 ml. and 0.0005 ml. of virulent culture, and one of the two ewes inoculated with 0.0001 ml. of culture died.

In the group of six ewes immunized with 5 ml. of the formalinized broth culture vaccine and challenged with virulent culture four weeks later, one of four ewes survived the inoculation of 0.5 ml. of culture, and two ewes survived the inoculation of 0.25 ml. of culture.

Sheep vaccinated with 5 ml. of the alum-precipitated product were protected against 10 ml. and 11 ml. of virulent culture, and a further two of three ewes survived the inoculation of 12 ml. of culture.

The results of this trial indicated that a highly potent *Cl. chauvoei* vaccine could be prepared in tissue-free broth, particularly when the product was alum-precipitated in addition.

Development of an Improved Type of Cl. chauvoei Vaccine

The preliminary tests with vaccine prepared from a tissue-free broth culture indicated that an efficient immunizing agent for sheep could be prepared in this manner. Another practical advantage of the tissue-free broth culture vaccine was the considerable simplification in the method of production. Consequently it was decided to concentrate attention on the development of a tissue-free vaccine which could be prepared conveniently on a large scale, and which would be alum precipitated to further enhance its antigenicity.

In the course of the development of the blackleg vaccine, a number of modifications in the technique of preparation were tested against the original procedure outlined above. In many instances the experiments were repeated on numerous occasions, and the conclusions from these tests are summarized here.

1. *Type of Flasks*: Rajagopalan (1947) advocated the use of conical flasks filled to the neck for the growth of *Cl. chauvoei* in the cysteine broth. Apparently the principle behind this practice was to reduce the surface of the medium exposed to the aerobic atmosphere. Growth was compared in conical and florence flasks and no significant difference was apparent.

2. *Volume of Medium*: In the original studies by Rajagopalan (1947), the experimental observations were conducted with small volumes of medium, in fact the largest amount to which reference was made was 200 ml. In the Wallaceville studies satisfactory growth was observed in broth of volumes up to 20 l. With volumes of broth over 5 l., autoclaving was conducted at 15 lb. pressure for 30 min. to ensure sterility. Apparently the extra period of autoclaving had no significantly detrimental effect on growth.

3. *Reaction of Medium*: The effect of an initial pH of the medium over a range from 6.0 to 8.0 prior to autoclaving was determined. Growth was observed with all media of initial pH prior to autoclaving of 6.0 to 8.0, with an optimum between 7.2 and 7.4. In media with a pH above 7.2, a small deposit of phosphates was observed after autoclaving, but this was not considered to be of any consequence.

4. *Period of Incubation*: Growth was usually well developed after 10 to 12 hr. incubation and continued, if flasks were not violently agitated, until the second day of incubation. Measurement of growth from cultures incubated for one day, two days and four days, indicated that all significant growth was completed after 48 hr. incubation.

5. *Type of Broth*: Growth was compared in horse muscle broth, beef broth and "V.F." broth (peptic digest of liver and beef), to each of which 0.05 per cent cysteine and 0.5 per cent glucose had been added. Growth of *Cl. chauvoei* was comparable in all three media. Meat-extract broths prepared from American and British proprietary meat extracts were significantly inferior in supporting growth as compared to beef-infusion broth.

6. *Types of Peptone*: Growth was compared in beef infusion glucose cysteine broth prepared with six different proprietary peptones and no significant difference was observed. Concentration of peptone could not be reduced below one per cent without significant influence on total concentration of bacterial cells. ("V.F." broth does not require the addition of commercial peptone).

7. *Addition of Yeast Extract*: Addition of 0.3 per cent yeast extract to the standard broth resulted in no significant improvement in cell concentration.

8. *Strain of Cl. Chauvoei*: A recently isolated ovine strain of *Cl. chauvoei* grew satisfactorily in the beef-infusion glucose cysteine

broth. The strain of *Cl. chauvoei* used throughout these studies was of bovine origin and has been used for vaccine production in New Zealand over the past sixteen years.

9. *Reducing Substances*: Contrary to the experience of Rajagopalan (1947), the Wallaceville vaccine strain of *Cl. chauvoei* grew profusely in the beef-infusion glucose broth in the absence of cysteine. The influence of other reducing substances such as ascorbic acid, sodium thioglycollate and thioglycollic acid in place of cysteine in supporting the growth of *Cl. chauvoei* in the standard beef-infusion glucose broth was examined in a series of experiments. The following results were observed in one of the experiments conducted on this aspect of the problem:

Medium	Weight of Dried Cells (Mg. per 100 ml. Culture Broth)
1. Beef infusion glucose broth	53
2. Beef infusion glucose broth + 0.05 per cent cysteine HCl	65
3. Beef infusion glucose broth + 0.1 per cent ascorbic acid	57
4. Beef infusion glucose broth + 0.1 per cent sodium thioglycollate	56
5. Beef infusion glucose broth + 0.02 per cent thioglycollic acid	58

Cysteine HCl compared consistently favourably with the other reducing agents tested in enhancing the growth of *Cl. chauvoei* in the beef-infusion glucose broth.

10. *Effect of Neutralization and Addition of Glucose during Incubation*: It was observed that during the growth of *Cl. chauvoei* in the beef-infusion glucose cysteine broth, the pH decreases to the vicinity of 5.0, whereupon growth gradually ceases. A series of experiments were undertaken to determine the influence of adjusting the pH to 7.0, after one or two days' incubation, and then continuing incubation for a further one or two days. Also studied were (1) the effects of adding 0.5 per cent glucose after one or two days' incubation, and continuing incubation, and (2) combining the adjustment of the pH to 7.0 and adding glucose after one or two days' growth and continuing incubation. The results of these experiments indicated that adjusting the pH after one or two days' growth resulted in a further development of rapid growth associated particularly with vigorous gas formation. The addition of glucose alone did not result in further growth nor did the addition of glucose at the time of adjustment of the pH to 7.0 enhance the effect of the neutralization.

The following results were observed in one experiment to determine the influence on the growth of *Cl. chauvoei* in the standard broth with and without cysteine, when the pH of the media was adjusted to 7.0 after 24 hr. growth and incubation then continued for a further 24 hr.

Medium	Incubation Procedure	Weight of Dried Cells (Mg. per 100 ml.)
Beef infusion glucose broth	48 hr. incubation.	52.9
Beef infusion glucose broth + *cysteine*	48 hr. incubation.	74.1
Beef infusion glucose broth	pH adjusted to 7.0 after 24 hr., then incubated for further 24 hr.	87.5
Beef infusion glucose broth + *cysteine*	pH adjusted to 7.0 after 24 hr., then incubated for further 24 hr.	92.2

It will be observed from these results that neutralization during incubation had a most significant influence on the final concentration of bacterial cells in the two media. The results might suggest that if *Cl. chauvoei* was grown for 48 hr. in a beef-infusion glucose medium without cysteine and the pH of the medium were adjusted to 7.0 after 24 hr. incubation, the concentration of bacterial cells and possibly its efficiency as an immunizing agent might be superior to a vaccine prepared by the original technique. However a practical disadvantage with this procedure is that the adjustment of the reaction during growth could provide an opportunity for the contamination of the broth culture if adequate precautions were not provided. Observations were made on the effect of the addition of a quantity of fine marble chips to the beef-infusion glucose-cysteine medium in delaying the fall in the pH of the broth culture and so possibly increasing the concentration of bacterial cells. No significant improvement in growth was observed with this treatment. The addition of dibasic alkali phosphate to the standard broth may exert some beneficial effect in the total bacterial concentration in the resultant vaccine.

11. *Stage of Growth in Inoculum and Temperature of Medium when Inoculated*: It has been demonstrated repeatedly that if the inoculum does not contain rapidly proliferating organisms and the temperature of the medium is considerably below 37° C. at time of inoculation, the resultant growth is not satisfactory. The influence of these factors became progressively accentuated when inoculating large volumes of medium. Consistently satisfactory results have been obtained by ensuring that the flasks of medium are at approximately 40° C. when seeded with inoculum which is gasing profusely. Ten-to-eighteen-hour cultures have been observed to be most satisfactory for use as inocula.

12. *Modified Procedures for the Preparation of Beef Infusion Broth*: The effects of a number of modifications in the technique of preparing the standard beef-infusion glucose-cysteine broth were determined in an endeavour to develop a more convenient procedure for the large scale routine manufacture of vaccine.

No significant reduction in growth was detectable in broth prepared without soaking the minced beef in cold water overnight in the cold room. In the preparation of beef-infusion broth by the original procedure, the infusion is heated on two occasions prior to the final sterilization. Considerable simplification of the procedure was found possible, without detriment to the nutritive quality of the broth or of the antigenic efficiency of the vaccine as assessed by sheep immunity tests, by eliminating the second heating process. The modified procedure finally adopted involved the addition of 10 N.NaOH solution to adjust the pH of the infusion to 7.0 during the first heating process which facilitated the precipitation of proteins and subsequent separation of the broth.

13. *Formalinization and Alum Precipitation*: With the adoption of the simplified procedure for the preparation of the beef-infusion broth, it was decided to increase the quantity of formalin, which is added to the culture to effect sterility, from 0.5 to 0.8 per cent. This was considered advisable in view of the increased quantity of precipitated proteins occurring following growth in the medium prepared by the modified technique, since the presence of this protein could result in inactivation of some of the formalin. Incubation was continued as previously for 24 hr. after formalinization.

For convenience in the large scale production of blackleg vaccine, alum precipitation was conducted with the anaculture and potash alum solution at room temperature. A 20 per cent sterile alum solution is added to the anaculture to make a final concentration of 2 per cent alum.

A series of experiments were undertaken to study the type of precipitation which resulted when the alum solution was added to anacultures of various pH. The series of photographs illustrate the type and rate of precipitation resulting when the 20 per cent alum solution was added at room temperature to a variety of anacultures.

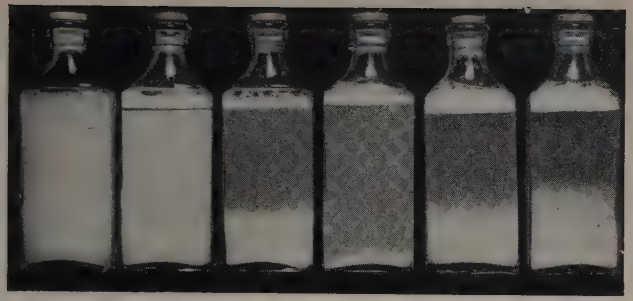
The photographs (Fig. 1) illustrate the appearance of the vaccines at 1 min., 5 min., 1 hr. and 18 hr. after the addition of the concentrated potash alum solution.

The anacultures in order from left to right are:

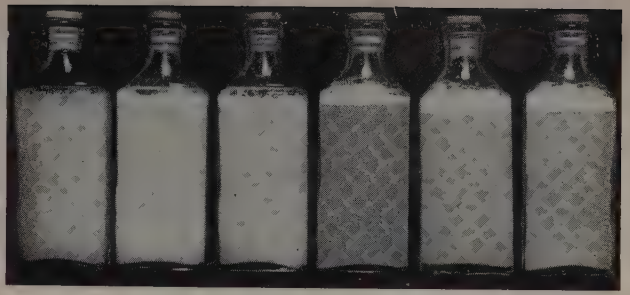
(1) Single heating process, beef-infusion glucose-cysteine (B.I.G.C.) anaculture pH 5.0;

(2) Single heating process, B.I.G.C. anaculture pH adjusted to 7.0 prior to formalinization;

(3) Single heating process, B.I.G.C. anaculture pH adjusted to 7.0 after formalinization;



B. At five minutes.



A. At one minute.

D. At eighteen hours.

C. At one hour.

FIG. 1.—Alum Precipitation of Blackleg Vaccines. Nature of precipitate in anacultures after addition of potash alum solution.

(4) Double heating process, B.I.G.C. anaculture pH 5.0;

(5) Double heating process, B.I.G.C. anaculture, pH adjusted to 7.0 prior to formalinization;

(6) Double heating process, B.I.G.C. anaculture, pH adjusted to 7.0 after formalinization.

It will be observed with media prepared by both the single and double heating procedures, when the pH of the broth cultures was not adjusted to 7.0, precipitation was slow and was not complete after standing for 18 hr. In the case of the medium prepared by the single heating procedure, when pH of the culture was adjusted to 7.0 prior to formalinization, the precipitate was of a fine floccular type which deposited slowly to leave a clear supernatant. With similarly prepared medium, when the pH of the culture was adjusted to 7.0 after formalinization, the precipitate was composed of larger types of floccules which deposited more rapidly than in the previous case, but precipitation was not complete after 18 hr. as evidenced by the cloudy supernatant.

Using medium prepared by the original double heating procedure there was no obvious difference in the nature or rate of precipitation when the pH of the culture was adjusted either prior to or following formalinization. In both cases precipitation was complete after 18 hr. The nature of the precipitation under varying conditions is of considerable practical significance when bottling is undertaken after precipitation or where efforts are to be made to concentrate vaccine by removal of the supernatant fluid, if loss of antigenic material is to be avoided. Further, it is desirable that the precipitate in a vaccine should be of a type which will disperse readily again by shaking after remaining undisturbed for protracted periods.

Technique finally adopted for large scale preparation of Cl. chauvoci Vaccine

The following technique has been shown to be suitable for the large scale production of blackleg vaccine by the preparation at Wallaceville when the necessity arose of 1,000 l. (200,000 doses) of vaccine in a period of two weeks:

Finely minced lean beef is added to tap water at the rate of 500 g. of beef to 1 l. of water. Mixture is well stirred and gradually heated to boiling point. Reaction of the boiling infusion is adjusted to pH 7.0 by the addition of 10 N NaOH solution. Mixture is simmered with regular stirring for 30 min. After cooling, meat infusion broth is separated from sedimented tissue and held temporarily at 4° c. Clear broth is siphoned off from consolidated fat and sedimented precipitate. Tap water is added to infusion broth to adjust to the original volume.

One per cent peptone, 0.5 per cent sodium chloride and 0.05 per cent cysteine HCl are added and pH is adjusted to 7.4 with 10 N NaOH solution.

Broth is distributed into pyrex flasks and sterilization is effected by autoclaving at 15 lb. pressure for 20 min. for volumes up to 5 l. and 15 lb. pressure for 30 min. for volumes of 10 to 20 l. After autoclaving, media in flasks are cooled rapidly to 40° C.

A 2–4 per cent volume of a 10–15-hr. culture of *Cl. chauvoei* (Wallaceville vaccine strain Cl. 7) in beef-infusion glucose-cysteine broth is used as the inoculum.

Flasks are incubated at 37° C. for 48 hr. The pH of the culture is adjusted to 7.0 and 0.8 per cent formalin is added. Sterile rubber bungs replace the cotton-wool plugs in the flasks and incubation is continued for a further 24 hr.

Prior to formalanization, the culture is surface sown on blood-agar plates which are incubated aerobically and anaerobically for 48 hr. as a check on the purity of the culture. After the formalinized broth culture has been incubated for 24 hr., the sterility of the vaccine is confirmed by inoculating samples into tubes of sheep heart medium which are then incubated for three to four days and by inoculating guinea pigs intramuscularly with 5.0 ml. of the product.

After the bacteriological and biological tests have confirmed the sterility of the formalinized broth culture, alum precipitation is conducted by the addition at room temperature with vigorous agitation of sterile 20 per cent potash alum solution to make a final concentration of 2 per cent alum. The reaction of the alum precipitated vaccine is then adjusted to pH 7.0 by the addition of 10 N. NaOH solution. The vaccine is bottled with regular agitation of the bulk supply.

Two sheep which have not been immunized previously are inoculated subcutaneously with 20 ml. of a sample of vaccine from each batch, as a confirmatory test of the innocuity of the vaccine.

Potency Testing of Blackleg Vaccine

Since 1948, periodic tests for potency of batches of blackleg vaccine used in New Zealand, have been conducted at Wallaceville. Since sheep were used exclusively as the experimental animals in these tests, a minimum number of animals for reasons of economy was utilised on each occasion. Experimental evidence was available that indicated that sheep of twelve months of age or older, vaccinated with 5 ml. of the improved Wallaceville Blackleg Vaccine would survive the intramuscular inocuation of a 5 ml. dose of virulent culture when exposed to this artificial infection between two to six weeks following immunization. Non-vaccinated sheep were susceptible to doses of from 0.01 ml. to 0.0001 ml. of the virulent culture. Usually in a potency test of samples of vaccines, a group of sheep were included which had been immunized with vaccine prepared under standardized conditions at Wallaceville. In these tests two to four sheep were immunized with 5.0 ml. doses of each vaccine and after a minimal interval of two weeks, these animals were inoculated with doses of

5 ml. and greater of virulent culture. The vaccine was considered satisfactory if the immunized sheep survived a challenge dose of 5 ml. of the virulent culture. A minimum of four non-vaccinated animals was included in each test to confirm the virulence of the cultures used for artificial infection, the usual inocula being 0.0001 ml., 0.001 ml. and 0.01 ml.

Immunity produced in Sheep with Experimental and Commercial Vaccines

An experiment was conducted in August 1950, to compare the immunity induced in sheep by using samples of four commercial blackleg vaccines and the Wallaceville vaccine. The commercial vaccines tested included an American alum precipitated product, a blackleg

TABLE III. COMPARISON OF POTENCY OF VACCINES IN PRODUCING IMMUNITY IN HOGGETS AGED APPROXIMATELY 12 MONTHS.

(Animals challenged 4 weeks after vaccination; tested August, 1950)

Treatment	Dose of Virulent Culture (ml.)	Number Inoculated	Number of Survivors
2 ml. Wallaceville vaccine	0.5	1	1
	1.0	1	1
	2.0	3	1
	3.0	1	0
5 ml. Wallaceville vaccine	5.0	1	1
	8.0	2	1
	10.0	2	0
5 ml. N.Z. Commercial vaccine	5.0	1	1
	8.0	3	1
	10.0	3	0
5 ml. American Commercial vaccine	8.0	2	2
	10.0	4	1
5 ml. Australian Commercial vaccine	0.01	1	1
	0.1	1	0
	0.5	1	0
	1.0	1	0
5 ml. English Commercial vaccine	0.05	1	0
	0.1	1	0
	0.5	1	0
	1.0	1	0
Controls	0.0001	4	3
	0.0005	4	2

vaccine prepared and in current use in Australia, an English blackleg vaccine, and a product prepared in a commercial veterinary laboratory in New Zealand in accordance with the described Wallaceville procedure. The Wallaceville vaccine was a sample of a batch of vaccine which had been prepared for use in a controlled field trial. Groups of hoggets aged approximately twelve months were immunized with a 5 ml. dose of the four commercial vaccines, and with 2 ml. and 5 ml. doses of the Wallaceville vaccine. The vaccinated hoggets and a group of unvaccinated animals were exposed to artificial infection with virulent culture four weeks later.

The results of this trial are presented in Table III. They indicated that the Wallaceville, the New Zealand commercial and the American vaccines were potent products whilst the Australian and English vaccines, neither of which were alum precipitated products, were significantly inferior.

In Table IV, the results of an immunity test conducted in sheep with two commercial vaccines and a Wallaceville product are presented. This test was undertaken in November 1951, and the vaccines used included the improved type of vaccine prepared at Wallaceville, a blackleg vaccine prepared by the same technique in a New Zealand commercial vaccine laboratory, and a concentrated and combined blackleg and enterotoxaemia vaccine prepared in the same laboratory. Hoggets aged approximately fourteen months were vaccinated with 5 ml. doses of each product and the vaccinated and untreated animals were exposed to experimental infection two weeks later.

The results in Table IV would indicate that the two blackleg vaccines were of similar efficiency.

TABLE IV. TEST OF POTENCY OF BLACKLEG VACCINES IN PRODUCING IMMUNITY IN HOGGETS AGED APPROXIMATELY 14 MONTHS.

(Animals challenged 14 days after vaccination; tested November, 1951)

Treatment	Dose of Virulent Culture (ml.)	Number Inoculated	Number of Survivors
5 ml. Wallaceville vaccine	5.0	1	1
	8.0	2	1
5 ml. N.Z. Commercial blackleg vaccine	5.0	1	1
	8.0	2	1
5 ml. New Zealand Commercial, Combined and Concentrated blackleg-enterotoxaemia vaccine	5.0	1	1
	8.0	2	2
Controls	0.0001	2	1
	0.001	3	0
	0.01	2	0

This test supplied confirmatory evidence that blackleg vaccine could be concentrated and combined with other antigens without detriment to its efficiency.

The one strain of *Cl. chauvoei* of bovine origin has been used for vaccine production in New Zealand for sixteen years. It appeared desirable to compare the efficiency of vaccine prepared with the usual attenuated strain with a product prepared in an identical manner but using a freshly isolated and virulent strain of ovine origin. These two vaccines were tested in groups of four ewes. Three commercially prepared vaccines were included in the same test conducted in July 1953. These vaccines were a New Zealand commercially prepared blackleg vaccine (Wallaceville technique), a New Zealand commercial concentrated, and combined blackleg and enterotoxaemia vaccine, and an American alum precipitated blackleg vaccine. The results of this test presented in Table V indicated that efficient vaccines for the prevention of *Cl. chauvoei* infection in sheep could be prepared from

TABLE V. TEST OF POTENCY OF BLACKLEG VACCINES IN PRODUCING IMMUNITY IN ROMNEY EWES.

(Animals challenged 3 weeks after vaccination; tested July, 1953)

Treatment	Dose of Virulent Culture (ml.)	Number Inoculated	Number of Survivors
5 ml. Regular blackleg vaccine prepared at W.A.R.S.	1.0	1	1
	5.0	1	1
	10.0	1	1
	15.0	1	1
5 ml. vaccine prepared from virulent strain of *Cl. chauvoei*	1.0	1	1
	5.0	1	1
	10.0	1	1
	15.0	1	1
5 ml. New Zealand Commercial blackleg vaccine	1.0	1	1
	5.0	1	1
	10.0	1	0
	15.0	1	1
5 ml. Concentrated New Zealand Commercial combined blackleg-enterotoxaemia vaccine	1.0	1	1
	5.0	1	1
	10.0	1	0
	15.0	1	1
5 ml. American Commercial blackleg vaccine	1.0	1	1
	5.0	1	1
	10.0	1	1
	15.0	1	1
Controls	0.0001	2	1
	0.001	1	0
	0.01	1	0

isolated virulent ovine strain. The practical advantages associated with the use of the regular vaccinal strain would favour its retention for routine vaccine production.

Production and Persistence of Immunity in Hoggets

In the Otago and Southland districts in particular, losses of hoggets from *Cl. chauvoei* infection during winter feeding on root crops, constitute a serious problem. On properties where these losses of from either the regular laboratory attenuated bovine strain or a recently two to ten per cent recur annually, hoggets require to be vaccinated against blackleg, usually during the months of April or May. Quite regularly on the same properties, losses in two-tooth ewes from *Cl. chauvoei* infection following lambing may approximate five to ten per cent with a lower but significant percentage mortality in the older ewes from the same infection. Controlled field trials with the improved type of blackleg vaccine had shown that these losses in ewes could be effectively controlled by vaccination of the ewes during pregnancy.

An experiment was undertaken to determine the degree of immunity induced in hoggets aged approximately between seven and eight months following inoculation with 2 ml. and 5 ml. doses of the improved blackleg vaccine. The vaccinated sheep were challenged by the intramuscular inoculation of virulent culture at periods of 6 weeks, 32 weeks and 72 weeks after vaccination. Groups of non-vaccinated sheep of equivalent age were similarly exposed on the same occasions to check the virulence of the inoculated culture.

The results of this trial are presented in Table VI.

TABLE VI. PRODUCTION AND PERSISTENCE OF IMMUNITY IN ROMNEY HOGGETS AGED 7-8 MONTHS.

(Vaccine prepared in tissue-free broth: Alum precipitation)

Treatment	Challenge 6 weeks Post Vaccination			Challenge 32 weeks Post Vaccination			Challenge 72 weeks Post Vaccination		
	Dose (ml.)	No. Inoculated	No. Survivors	Dose (ml.)	No. Inoculated	No. Survivors	Dose (ml.)	No. Inoculated	No. Survivors
2 ml. Vaccine	0.05	4	2						
	0.1	2	0						
	0.5	2	0						
	1.0	2	0						
5 ml. Vaccine	7.0	2	2	4.0	1	1	0.5	3	1
	8.0	5	5	5.0	1	1	1.0	3	2
	9.0	5	2	6.0	4	4	2.0	3	0
	10.0	2	0	7.0	2	1	5.0	1	0
				8.0	2	0			
Controls	0.0001	1	1	0.0001	1	1			
	0.0005	3	2	0.0005	3	2	0.0005	4	2
	0.001	2	0	0.001	3	1	0.001	3	1

The results from this experiment were of considerable interest. They suggested that whilst a dose of 2 ml. of the improved vaccine induced a satisfactory response in mature sheep, young sheep of seven to eight months of age responded inadequately to the same treatment. However, similar young sheep did exhibit a satisfactory degree of immunity when the same vaccine was administered in a dose of 5 ml. It is of interest to compare the results in Table VI with those summarised in Table III which indicate the significantly higher immunity response, to a 2-ml. dose of vaccine, of the 12 months old hoggets as compared with those 7–8 months old.

The experiment indicated, further, that hoggets immunized with a 5 ml. dose of vaccine, during the month of April, still exhibited a satisfactory degree of immunity when tested eight months later. After an interval of 72 weeks, following vaccination, the immunity of the sheep had deteriorated significantly. These observations would suggest that ewes vaccinated as hoggets before being put on to winter feed, and likely to be exposed to gross infection at time of first lambing as two-tooths, should be revaccinated during their first pregnancy.

Progressive Application of Vaccination

Following the recognition in 1938 of the role of *Cl. chauvoei* in sheep mortalities in New Zealand, 4,800 sheep were experimentally vaccinated against this infection in 1939. An increasing demand in subsequent years for the free issue of blackleg vaccine from the Wallaceville Animal Research Station was the logical consequence of an increasing appreciation of the prevalence and economic importance of *Cl. chauvoei* infection in sheep in New Zealand and the value of vaccination in reducing these losses. Vaccination of sheep for blackleg was confined to the North Island until 1945, and sheep were not vaccinated in Otago or Southland until 1947. From 1943 to 1947 inclusive, approximately 30,000 doses of blackleg vaccine for the inoculation of sheep were issued from Wallaceville annually. The number of sheep immunized against blackleg in New Zealand increased to 180,000 in 1948, and of this total, 73,000 animals were located in Otago and Southland.

The demand in 1949 for blackleg vaccination of sheep increased at a similarly spectacular rate, the number of doses of vaccine issued for sheep being 398,650 for a twelve months' period. Of this total, 125,050 doses were used in the North Island and 273,600 in the South Island, including 202,050 doses in the Southland county.

The improved type of vaccine was first issued from Wallaceville for general field use in 1949. In March 1950 the free distribution of blackleg vaccine from Wallaceville for sheep immunization was terminated. In view of the uniformly satisfactory results with the improved vaccine in controlled field trials on properties where heavy mortalities were experienced regularly, it was desirable that commercially prepared vaccine to be used under similar conditions should be of equivalent efficiency. Consequently arrangments were made to ensure that commercially prepared blackleg vaccine for use in sheep in New Zealand was of high potency. All blackleg vaccine available commercially in New Zealand since 1950 has been prepared in accordance with the technique developed for the improved Wallaceville vaccine, and samples of vaccine have been subjected periodically to potency tests with sheep, as a check of the efficiency of the products.

Recommendations for Use of Improved Vaccine under Field Conditions

Recommendations for the most effective use of the vaccine for the control of *Cl. chauvoei* infections of sheep under field conditions have been suggested following consideration of the results from immunity trials in sheep at Wallaceville and from controlled vaccination experiments involving heavy exposure to natural infection on selected properties.

The recommended dose of vaccine for sheep of all ages is 5 ml. subcutaneously.

On properties experiencing mortalities in ewes from *Cl. chauvoei* infection following lambing, pregnant ewes should be vaccinated with the 5-ml. dose not later than three weeks prior to the anticipated date of lambing. In subsequent years, in general, vaccination of the pregnant two-tooth ewes only is necessary. On properties, where it has been established that *Cl. septicum* infection does contribute to sheep mortalities in addition to *Cl. chauvoei* infection, sheep should be immunized with a 5-ml. dose of concentrated and combined *Cl. chauvoei* -*Cl. septicum* vaccine.

Where loss of hoggets occurs from *Cl. chauvoei* infection during winter feeding on root crops or on pasture, hoggets should be immunized with a 5-ml. dose of vaccine three weeks prior to the expected date of exposure to infection. Mortalities in ram hoggets from *Cl. chauvoei* infection of traumatic injuries can be prevented by similar treatment.

Two-tooth ewes immunized as hoggets require a second inoculation of 5 ml. of vaccine during pregnancy to protect against *Cl. chauvoei* infection at lambing.

Mortalities in lambs from *Cl. chauvoei* infection at docking and castration, on properties where such mortalities recur regularly, may be prevented by vaccination of the ewes three weeks prior to lambing and by docking the lambs not older than three weeks of age. Artificial exposure of experimental lambs has shown that prenatal vaccination of the ewe does confer immunity on the young lamb. Ewes would require to be revaccinated annually with a 5-ml. dose three weeks prior to lambing, where protection of future crops of lambs against infection at docking has been shown to be necessary.

Where a sporadic mortality from *Cl. chauvoei* infection has occurred in portion of an unvaccinated flock following shearing, crutching or dipping, prevention of further mortalities can be effected by immediately vaccinating the remainder of the flock and delaying the operations for two weeks.

Acknowledgments

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LABOUR-SAVING DEVICES IN SOIL TESTING

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Summary

Some labour-saving devices used in handling large numbers of soil samples are depicted and described.

Introduction

Soil analysis is now a recognised aid to the Extension Officer in his work, and the demand for analyses has expanded very greatly. By the adoption of various labour-saving devices, it has been possible to increase output fourfold without increasing laboratory staff. These devices, which present original features, are simple in design and should be within the reach of any establishment with a glassblower and a small workshop.

Description of Apparatus

1. *Soil Measure*

The soil measure shown in Fig. 1 has proved very satisfactory and ensures that similar volumes of soil are taken for each determination.

Fig. 1.—Soil Measure

The measure is constructed with a false top which can be swung to one side by light pressure on the handle. When filling the measure, top and bottom portions are in line and form a continuous cylinder. After filling, the measure is tapped down two or three times on the

bench, and then the surplus soil wiped off by compression of the handle. A light spring in the hinge returns the top portion into place when pressure is released. This measure has proved more rapid in action and has given better reproducibility than the measuring spoon which is still in general use in United States soil testing laboratories[1].

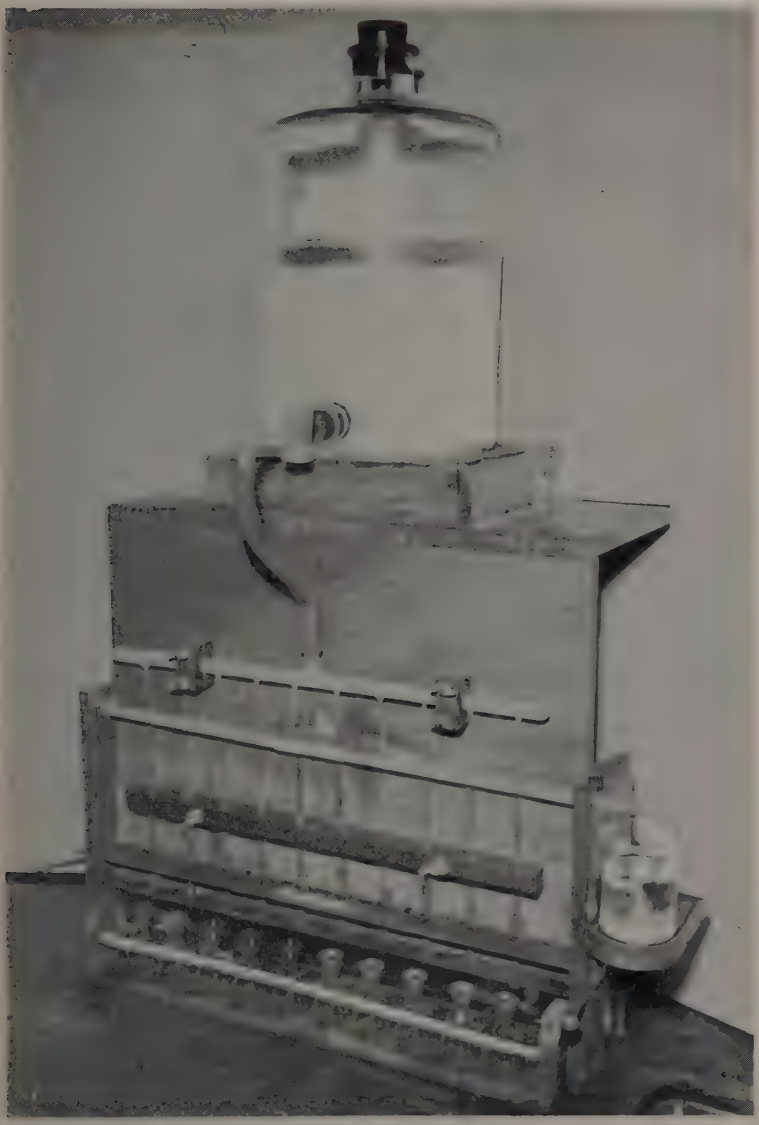

FIG. 2.—Dispensing Unit

2. *A 10 ml. Dispensing Unit* (Fig. 2).

Simultaneous addition of extractant is accomplished by means of the apparatus shown. An aspirator connected to a glass manifold provides an overhead supply to a series of glass tubes. These are connected at the base to short pieces of rubber tubing and cut-off is accomplished by tension of a spring-loaded brass bar against the rubber tubes.

The bore of the aspirator tap must be large enough to allow rapid filling of the manifold, while the manifold jets are constricted at the tips so that the rate of flow of liquid is approximately equal from each. The glass tubes—internal diameter about 5/16 in.—have an overflow hole ½ in. from the top. They then pass through an inclined perspex trough which carries away the surplus reagent. Small pieces of rubber tubing around the glass tubes where they pass through the perspex ensure a good seal. The tubes can be easily calibrated by varying the distance they project into the rubber tubing below.

To use the apparatus, the aspirator tap is turned on till all tubes are overflowing. Depression of two levers, one at each end of the wooden frame, then eases the brass bar away from the rubber tubes, releasing reagent into the flasks below.

This apparatus, having an overhead source of supply, is simpler in design and hence cheaper and easier to construct than the batteries of automatic pipettes used by some overseas soil testing laboratories[2].

3. *A Simple Flask Shaker* (Fig. 3)

This is a cradle type shaker driven by a small motor with built-in reduction gear, giving a final speed of 140 r.p.m. The rack of flasks, which needs no clamping, is placed sideways in the cradle, and time of shaking is regulated by a time switch. Bench space required is only 30 in. by 12 in.

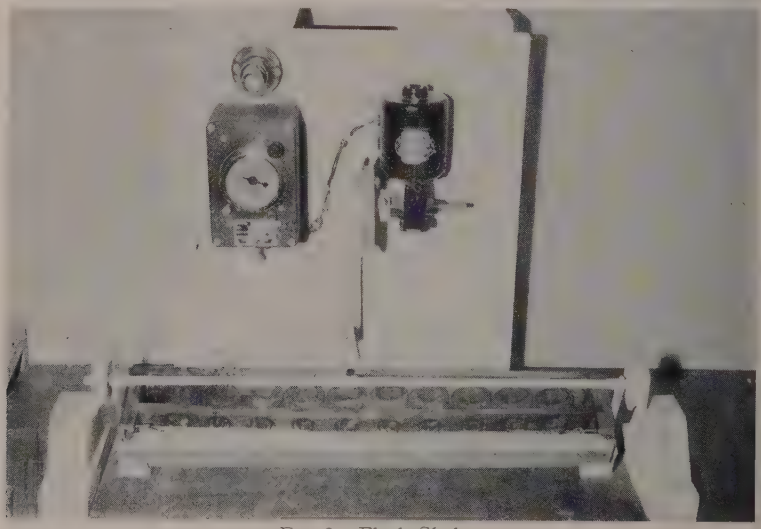

Fig. 3.—Flask Shaker

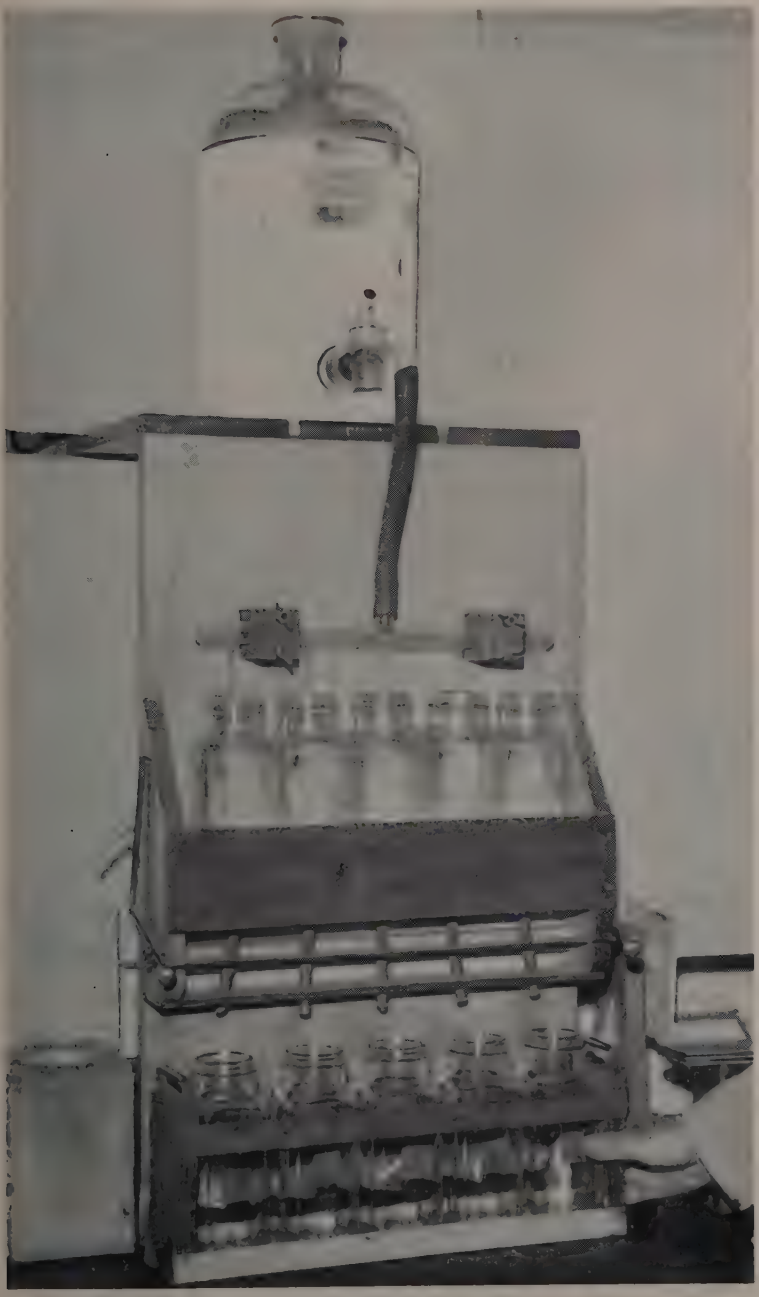

FIG. 4.—Dispensing Unit

4. *A 400 ml. Dispensing Unit* (Fig. 4)

This is similar in principle to the 10-ml. dispenser, but bottles are used to deliver the larger volumes employed. Each bottle has two ½-in. diameter holes bored in it, one centrally in the base, and another on one side, about ¾ in. above the 400-ml. mark. Connections to both holes are by means of glass tubing, sleeved at the ends by short pieces of rubber tubing, and fitting tightly into the bored holes. The overflow tubes pass through to the rear of the backing board, where they dip into holes blown in an inclined glass tube which carries the surplus reagent to a waiting beaker. The glass outlet tubes in the base are connected to rubber tubing, and cut-off is, as before, by pinchcock effect from a brass bar. Each jar is easily calibrated by raising or lowering in its rubber sleeve the glass tubing protruding through the base. The hollow stopcock of the aspirator has two small holes bored in it—one in the base and another in the barrel. In the "off" position, air is admitted to the manifold to speed up draining.

5. *Tipping and Filtering Units* (Fig. 5)

Because of the relatively large amounts of solution employed in the phosphate test, the usual hand inversion of the extraction flasks over the filter funnels was impracticable. The use of the tipping units shown, reduced lifting to a minimum. All bottles were treated with a silane derivative (D.C. 200 Dow-Corning Corporation). As a result, they pour cleanly with no tendency to dribble back down the sides.

Fig. 5.—Tipping and Filtering Units

The top of the filter stand is easily lifted off, being loosely dowelled at each end, while the central strip of plywood can also be removed by turning two thumbscrews through 90°. (The centre piece of wood merely serves to hold the flasks in place when the rack is later inverted over the washing jets.)

6. *Adjustment of Volumes*

The present phosphate method requires that each flask contain 50 ml. of solution before proceeding to the final step of colour development. This is achieved by allowing filtration to proceed till the flasks are nearly full, and then removing excess solution with the apparatus shown in Fig. 6.

FIG. 6.—Apparatus for removing excess solution from flasks

A series of fine-tipped tubes, connected to a filter pump, is lowered into the flasks and the pump turned on, the length of the tubes being such that 50 ml. of filtrate remain in each flask. It is necessary to select flasks for uniformity. A filter pump with a high rate of evacuation is desirable. If it is found that the capacity of the pump is inadequate, a filter flask and stop-cock (large diameter bore) can be inserted in the line, and the vacuum built up beforehand. The suction apparatus is washed by lowering the tubes into a trough of water, with the pump still operating.

7. *A Rapid Water Dispensing Unit* (Fig. 7)

In the preparation of soils for pH measurement, some rapid method for measurement and delivery of constant volumes of distilled water is needed.

FIG. 7.—Water Dispensing Unit

The apparatus shown is essentially a table carrying five laboratory blowing balls of 2 in. diameter. Pressure of a hinged lid forces water into the soil beakers; release of pressure results in the automatic filling of the balls. Set screws in the lid allow adjustment of all bulbs to the approximate volume desired. Individual adjustment, necessary because of variation in capacities, is provided by resting each bulb on a small hinged platform carrying an adjusting screw at one end. In practice it has been found that once the initial calibration has been made, there is little need for any further adjustment. The rubber tubes for the water intake are gathered together into a stoppered jar which automatically fills from the aspirator above as liquid is withdrawn. With this apparatus, the addition of the distilled water can be accomplished in one fifth of the time previously taken.

The devices described have been in daily use in the laboratory for a number of years, proving reliable, and requiring very little maintenance.

Acknowledgments

Thanks are due to fellow staff members for suggestions in designs and assistance in making various pieces of apparatus, especially to Dr. E. B. Davies for helpful advice, Mr. I. C. Sweetman for the soil-shaker design and construction of apparatus, Mr. C. B. Wright for all glass work, and Miss B. C. Gee for the photographs.

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EFFECT OF PREGNANT MARE SERUM ON THE REPRODUCTIVE PERFORMANCE OF ROMNEY EWES

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Summary

The breeding performance of 282 Romney ewes injected on the twelfth or thirteenth days of the oestrous cycle with varying quantities of pregnant mare serum (P.M.S.) gonadotrophin has been compared with that of 235 untreated control animals, and the possible usefulness of gonadotropic treatment as a method of increasing fertility has been discussed in the light of results obtained.

The main findings were as follows:

(1) In animals treated with P.M.S. the onset of the next oestrus was slightly hastened.

(2) The proportion of ewes which held to first service was similar amongst P.M.S. treated and control animals.

(3) P.M.S. treatment did not result in any overall increase in the proportion of ewes which failed to conceive at all.

(4) Injection earlier than four days before oestrus, however, seriously impaired the chances of immediate conception.

(5) P.M.S. treatment led to a considerable increase in the number of lambs born. The increase was due almost entirely to a greater incidence of multiple births amongst those ewes that conceived at the oestrus which followed injection.

(6) The extent of the increase in the number of lambs born varied according to the age of the animals treated.

(7) Following treatment with 1,000 i.u. P.M.S., litters of up to six lambs were produced. When not more than 500 i.u. was used no litters larger than triplets were obtained.

(8) Associated with increase in litter size was a marked decrease in birth weight, such that the mean birth weight of lambs born as quadruplets was only approximately half that of those born as singles.

(9) The mortality rate was exceedingly heavy amongst lambs born in larger sized litters.

(10) In spite of the increased lamb mortality, final lambing percentages (lambs alive at 28 days as percentage of ewes mated), were increased as a result of P.M.S. treatment. In one trial where 1,000 i.u's. P.M.S. was used, an increase of 32 per cent was obtained.

Introduction

During the last twenty years a considerable amount of work has been done on the treatment of ewes with gonadotrophic hormones with a view to obtaining pregnancy during the period of normal anoestrus, advancing the onset of the breeding season, or inducing superovulation within the normal breeding season in order to increase fecundity. The extensive literature which now exists upon these subjects has been reviewed by Phillips, Fraps and Frank (1945), and more recently by Robinson (1951a). No tech-

nique so far employed in attempts to induce anoestrous ewes to breed or to advance the onset of the breeding season has been sufficiently successful to warrant adoption in commercial practice.

The position is much more promising in respect of augmenting the fecundity of ewes bred during the normal breeding season. Various workers have demonstrated that a considerably increased ovulation rate may be obtained as the result of a single dose of mare serum gonadotrophin injected during the follicular phase of the oestrous cycle. There is evidence that injection at an appropriate dosage level does not seriously upset the normal reproductive cycle, and that the eggs shed by treated ewes are capable of being fertilised and of undergoing further development. However, where many such artificially ovulated eggs are fertilised and commence development, a high proportion usually degenerate at early embryonic stages. The number of lambs produced at full term has not always exceeded that which might be expected from untreated animals, but in some instances very appreciable increases in lambing percentage have been secured.

In view of the differing results that have been obtained, more information is required as to the practical extent to which the number of lambs born and reared may be increased by gonadotrophic treatment at various levels carried out upon a flock basis. Particularly is there need for further data as to whether the ease of conception and hence the spread of lambing is affected, and as to the effect upon the incidence of ewes which fail either to become or to remain pregnant, and upon the extent of ewe mortality during pregnancy. These are questions which cannot be answered adequately by laboratory studies with a few animals, but only by large scale, carefully controlled, field trials. It is with the results of such trials, carried out during two separate seasons, that this paper is concerned.

Nature and Scope of Trials

The trials reported were conducted during 1951 and 1952. In the first season three separate trials (Trials I, II and III), involving respectively 197, 20 and 37 ewes, were carried out at the Ruakura Animal Research Station, situated in the Waikato district, while in the second season a single trial (Trial IV), with 263 ewes, was conducted at the Manutuke Research Station near Gisborne.

The purpose of the trials was to test the method of treatment that has been proposed by Robinson (1951) as worthy of field trial. The recommended technique consists of injecting ewes on the twelfth or thirteenth days of the oestrous cycle with sufficient gonadotrophic material to induce a moderate number of ovulations. A dose level considered suitable is one which will induce from 3 to 10 ovulations. Robinson, who worked with Cheviot X Border Leicester and Cheviot X Dorset ewes, obtained this ovulation response after injection of 500 i.u. of pregnant mare serum gonadotrophin (P.M.S.). However, results from a preliminary study (Wallace, 1953) have shown that approximately twice this quantity is required in order to obtain the same range of ovulations

in New Zealand Romney ewes. Accordingly, in the field trials of the first year, a dosage level of 1,000 i.u. was employed; in the second season a lower dose level, 500 i.u., was tried.

MATERIAL AND METHODS

(1) *Gonadotrophic material, method of administration, and dose levels employed*

Three separate batches of commercially prepared dried pregnant mare serum were used, two of "Antostab" (Batches A and B) (Boots Pure Drug Co. Ltd.), and one of "Serogan" (British Drug Houses Ltd.). These were used on the basis of their labelled potency, but each was subsequently checked against International Standard Preparation, using the increase in ovarian weight of immature female rats as the criterion of response. The "Antostab" of Batch A was found to be of labelled potency, but that of Batch B, which was very old material, proved to be only 20 per cent of labelled strength. The "Serogan" assayed at 87 per cent of stated potency.

In Trials I, II and III, the treated animals all received 1,000 i.u. of batch A "Antostab" which, as already indicated, was of full strength. In Trial IV, though the intention was to administer a dose of 500 i.u., only 57 of the 131 animals—those treated with the fully potent Batch A "Antostab"—received the complete dose. Fifty-nine were treated with "Serogan" and 15 with Batch B "Antostab". These latter animals actually received 435 i.u. and 100 i.u. respectively.

In all trials the ewes were given the material in aqueous solution as a subcutaneous injection. In Trials I, II and III, the injection was made in the region of the axillae, in Trial IV, in the area of the depression immediately behind the shoulder blade.

Information is available as to the efficacy of two of the batches of material in inducing superovulation in 6½-year-old Romney ewes. Part of the Batch A "Antostab" was used in a separate study (Wallace, 1953) to establish a dose-level — ovulation-response relationship, when it was found that animals injected with 500 i.u. and 1,000 i.u. on the twelfth and thirteenth days of the cycle had mean ovulation rates of 2.1 and 4.3 respectively. In a second investigation (Wallace, 1953), some of the "Serogan" was used. A dose of 650 i.u. gave mean ovulation rates of from 1.7 to 2.1 in different groups.

(2) *Experimental animals*

The ewes were all Romneys. The four flocks had each been reared on different properties and it is probable that strain differences existed between them. The age of the animals at the commencement of the trials varied. In Trial I, 100 of the ewes were 3½ years of age, and the remaining 97, 4½ years of age. The 20 ewes of Trial II and the 37 of Trial III were 6½ and 5½ years of age respectively. The 263 ewes of Trial IV were of three age groups: 74 were aged 3½ years, 111 were aged 4½ years, and 78 were aged 5½ years or more. All ewes were individually identified by numbered eartags.

(3) *Experimental procedure*

Trial I: In February, 1951, the 197 ewes were shifted from the Ruakura Hill Country Station at Whatawhata where the animals were reared, to Ruakura. Upon arrival at Ruakura they were run in a 3-acre paddock together with raddled, vasectomised rams. Very little pasture was available to the ewes and this was supplemented with hay. They were inspected daily and as each came into oestrus she was removed to a second paddock. There, every second animal within each age group was injected with 1,000 i.u. P.M.S., either twelve or thirteen days (rarely on the eleventh day) after her first oestrus. That half of the flock left untreated comprised the control group. Thirteen days after having been marked by the vasectomised rams, each ewe, whether treated or control, was transferred to a third field which contained fertile Romney rams. Tupping dates were again recorded and approximately a week after service by the fertile rams the ewes were returned to Whatawhata where both treated and control animals were run together as a single flock. At lambing time careful records were kept, both mother and offspring being caught and identified upon the day of birth. All deaths were noted. At docking time, when the majority of the lambs were about a month old, a check was made of those still alive.

Trials II and III: The procedure adopted in these two trials was essentially similar to that followed in Trial I, except that no animals were kept as controls. All animals received 1,000 i.u. P.M.S. either twelve or thirteen days after first oestrus. The fertile rams used were of the Southdown breed. The two flocks were farmed on separate areas. Adequate pasture was available and hay feeding was unnecessary.

Trial IV: This trial was conducted along lines similar to those of Trial I, with approximately equal numbers of treated and control animals. Injection was carried out every second day so that the treated animals received the P.M.S. either twelve or thirteen days after their first oestrus of the breeding season. Fertile rams of the Southdown breed were used.

Experimental Results

(1) *Oestrous cycle lengths*

Comparison of the cycle lengths of P.M.S. treated and control animals is possible in Trials I and IV. In Trial I there were 22 ewes in the control group and 17 in the treated group for which the date of service by the fertile rams was not recorded. In Trial IV rather more complete data was secured, service dates and hence oestrous cycle lengths being obtained for all but 5 control and 6 treated animals.

Since there was no evidence, in either Trial I or Trial IV, that the cycle lengths varied as between the age groups, these have been combined in Table I. The analysis of variance is presented in Table II. Owing to the coarse grouping (into whole days) and possibly non-normal distribution (positively skew), the analysis

TABLE I. EFFECT OF P.M.S. UPON OESTROUS CYCLE LENGTH

Trial	Treatment		No. ewes with cycle-length (in days) indicated									Total Ewes	Mean Cycle Length
			14	15	16	17	18	19	20	21	22		
I	P.M.S.	12th day	1	2	13	6	1					23	16.17
		13th day	3	6	17	20	2					48	16.25
		Total*	4	9	32	28	3	1				77	16.26
	Control			5	28	35	6	2	3	1	1	81	16.88
IV	P.M.S.	12th day		13	29	12	4		1			59	16.19
		13th day		11	30	18	6				1	66	16.38
		Total		24	59	30	10		1		1	125	16.29
	Control		1	7	50	59	6	3		1		127	16.60

*Total includes six animals injected on the eleventh day.

provides only approximate tests of significance. It does indicate, however, that the shortening effect of P.M.S. treatment on oestrous cycle length is highly significant (0.43 ± 0.10 days). Although the interaction (trials x treatments) does not attain significance, it is to be noted that the reduction in cycle length was greater in Trial I (0.62 days) than in Trial IV (0.31 days) where the lower dose was used. A relationship between the level of P.M.S. and the extent of the shortening of the cycle is thus suggested, although Robinson (1951) could find no such effect.

TABLE II. ANALYSIS OF VARIANCE OF CYCLE LENGTH (DAYS)

Source of Variation	d.f.	Mean Square
Trial I v. Trial IV	1	1.6
P.M.S. v. Control	1	18.8 ($P < .01$)
Interaction (trials x treatment)	1	2.3
Within subclasses	406	1.07

NOTE.—Allowance has been made in the above analysis for the unequal subclass numbers.

The data relating to the treated ewes have been further classified in Table I according to the day of injection. There is little evidence of any material difference in the time of onset of next oestrus following injection on the twelfth and thirteenth days respectively.

The above results are in general agreement with those of previous investigations. A hastened onset of oestrus following the administration of gonadotrophin during the follicular phase of the cycle has been observed by Warwick and Casida (1943), Laplaud and Thibault (1947), Robinson (1951), and Wallace (1953). Further, Wallace found little difference between the cycle lengths of animals injected on the tenth, eleventh, twelfth, thirteenth or fourteenth days.

(2) *Returns to service and proportion of ewes conceiving*

From the practical viewpoint, the proportion of ewes which conceive immediately, and the proportion of ewes which, though returning to service, ultimately become pregnant, are both of importance. P.M.S. treatment cannot be regarded as wholly satisfactory if it results in any substantial increase either in the spread of lambing or in the number of "dry" ewes.

The data are presented in Table III. There were a few ewes for which the relevant data was not obtained, as a result of the animals either dying or losing their identification tags.

TABLE III EFFECT OF P.M.S. UPON NUMBERS OF EWES CONCEIVING

Trial	Group	Number				Percentage		
		Pregnant		Non-pregnant	Total	Pregnant		Non-pregnant
		First Service	Later Services			First Service	Later Services	
I	Control	76	17	9	102	74	17	9
	P.M.S.	66	20	7	93	71	21	8
II	P.M.S.	11	7	2	20	55	35	10
III	P.M.S.	31	5	1	37	84	13	3
IV	Control	94	28	6	128	73	22	5
	P.M.S.	99	21	8	128	77	17	6
All Trials	Control	170	45	15	230	74	19	7
	P.M.S.	207	53	18	278	75	19	6

In Trial I, 74 per cent of the control ewes conceived as a result of first service by the fertile rams, whereas 71 per cent of the treated animals did so. In Trial IV, corresponding values for control and treated animals are 73 per cent and 77 per cent respectively. In each of the four trials the majority of the treated ewes which did not hold to first service became pregnant in a normal manner as a result of matings at subsequent uninjected heats, and in Trials I and IV where comparison is possible, the proportion of non-pregnant ewes is very similar for the control and treated groups. It appears that the conception rate was unaffected by P.M.S. treatment.

In Table IV the ewes (excluding those for which service data were not recorded) which returned to the rams have been classified according to the interval which elapsed between injection and first service.

With intervals of 4 days or less, a high proportion (148 out of 176, i.e., 84 per cent, conceived to first service. The proportion was significantly less ($\chi^2 = 4.5$, $P < .05$) where the interval exceeded 4 days (34 out of 49, or 69 per cent). These results substantiate findings already reported (Wallace, 1953).

TABLE IV. RETURNS TO SERVICE IN RELATION TO INTERVAL BETWEEN INJECTION AND OESTRUS
(Data for ewes which became pregnant)

Trial	Interval (days)	1	2	3	4	5	6	7	8	Total Ewes
I	Total	3	7	15	30	10	3	—	1	69
	Returns	2	—	3	4	4	1	—	—	14
II	Total	—	1	3	6	6	1	—	—	17
	Returns	—	—	1	3	2	—	—	—	6
III	Total	—	3	6	8	4	1	—	—	22
	Returns	—	—	—	2	1	—	—	—	3
IV	Total	—	11	37	46	18	3	—	2	117
	Returns	—	1	5	7	6	—	—	1	20
All Trials	Total	3	22	61	90	38	8	—	3	225
	Returns	2	1	9	16	13	1	—	1	43
	% Returns	12		15	18	34	18			

In Table V comparison is made of the numbers of ewes returning to service after treatment on the twelfth and thirteenth days respectively. In each case the percentage of ewes which conceived at first service was highly satisfactory (79 and 81 per cent).

TABLE V. RETURNS TO SERVICE IN RELATION TO DAY OF INJECTION
(Data for ewes which became pregnant)

Day of Treatment	12th day of cycle		13th day of cycle	
Trial	Treated	Returns	Treated	Returns
I	26	6	54	12
II	8	4	7	2
III	16	2	18	2
IV	57	10	61	10
All trials	107	22	140	26
% conceiving first service	79		81	
% returning	21		19	

(3) *Ewe mortality*

In Trial I, one control and one treated ewe died before lambing. In Trial II, two animals had to be killed; one was a very emaciated ewe with three foetuses, the other an animal which developed prolapse of the vagina after giving birth to quadruplets. In Trial III, a ewe which became extremely thin was found on slaughter to contain six foetuses. In Trial IV, no deaths occurred before lambing.

TABLE VI. LAMBING PERFORMANCE OF EWES

Trial	Group	Age (yr.)	EWES CONCEIVING TO FIRST SERVICE							EWES CONCEIVING TO LATER SERVICES				
			With Singles	With Twins	With Triplets	With Quads	Total Ewes	% with Multiple Births	Average Lambs per Ewe	With Singles	With Twins	Total Ewes	% with Multiple Births	Average Lambs per Ewe
I	Control	3½	36	3	—	—	39	8	1.08	8	1	9	11	1.11
		4½	35	2	—	—	37	5	1.05	8	—	8	—	1.00
		Total	71	5	—	—	76	7	1.07	16	1	17	6	1.06
	P.M.S.	3½	15	16	4	—	35	57	1.69	6	1†	7	14	1.29
		4½	13	13	2	3	31	58	1.84	10	3	13	23	1.23
		Total	28	29	6	3	66	58	1.76	16	4†	20	20	1.25
II	P.M.S.	6½	1	2	6	2	11	91	2.82	7	—	7	—	1.0
III	P.M.S.	5½	7	8	11	5*	31	77	2.52	5	—	5	—	1.0
IV	Control	3½	23	2	—	—	25	8	1.08	10	1	11	9	1.09
		4½	33	10	—	—	43	23	1.23	9	—	9	—	1.00
		Aged	20	6	—	—	26	23	1.23	6	2	8	25	1.25
		Total	76	18	—	—	94	19	1.19	25	3	28	11	1.11
	P.M.S.	3½	20	8	1	—	29	31	1.34	3	1	4	25	1.25
		4½	29	15	—	—	44	34	1.34	6	3	9	33	1.33
		Aged	14	12	—	—	26	46	1.46	4	4	8	50	1.50
		Total	63	35	1	—	99	36	1.37	13	8	21	38	1.38

*Includes one set of sextuplets. †Includes one set of triplets.

Of these deaths, only one, that of the ewe with sextuplets in Trial III, can be reasonably attributed to P.M.S. treatment. It is possible, also, that the deaths in Trial II were associated with the number of young produced by the two animals concerned.

(4) *The number of lambs born and the incidence of multiple births*

In Table VI the lambing data for ewes which conceived to first and to later services are separately shown. It is clear that P.M.S. treatment had a pronounced effect upon the lambing results.

(*a*) *Ewes which conceived to first service*: In Trial I, 58 per cent of the treated ewes had multiple births as compared with 7 per cent of the control animals. The difference is highly significant ($\chi^2 = 41$, $P < .01$) and is reflected in the numbers of lambs born per ewe, 1.76 for the treated as against 1.07 for the control ewes. In Trials II and III no control animals were available for comparison, but the incidence of large sized litters was much higher than commonly found amongst untreated animals. In each case triplets and quadruplets were produced, and there was one set of sextuplets. In Trial IV, where the smaller amount of P.M.S. was used, less striking results were obtained. Nevertheless, the proportion of multiple births was significantly greater in the case of the treated ewes (36 per cent as compared with 19 per cent of multiple births; $\chi^2 = 6.3$, $P < .01$). Neither in Trial I nor in Trial IV did any of the control animals produce more than two lambs.

It is to be noted that whereas amongst *control* animals the percentage of ewes with twins was significantly higher in Trial IV than in Trial I (19 per cent as against 7 per cent; $\chi^2 = 4.7$, $P < .05$); amongst *treated* ewes the reverse situation obtained (58 per cent with multiple births in Trial I compared with 36 per cent in Trial IV; $\chi^2 = 8.1$, $P < .01$). The level of nutrition during the breeding season was higher for the pasture fed ewes of Trial IV than for the hay supplemented animals of Trial I, which could account for the differential performance of the two control groups. The greater fecundity of the Trial I treated ewes is readily explained on the basis of the greater amount of P.M.S. employed.

It is of particular interest to note that the treated ewes of Trials I, II and III did not respond to the same extent, although all were injected with the same amount of P.M.S. The lambing rate was highest in Trial II, intermediate in Trial III, and lowest in Trial I (2.82, 2.52 and 1.76 lambs per ewe respectively). These differences are unlikely to be due to those which existed between the three flocks in respect of the way they were fed at the time of tupping, for Wallace (1953) has shown that the ovulation rate after P.M.S. injection is little influenced by the level of nutrition at that time. The effect of strain differences between the three lines of ewes cannot be ruled out; and it is possible also that the age of the ewes was an important factor. Ewes of four different age groups were represented in the three trials and, as may be seen in Table VI, the 3½-, 4½-, 5½-, and 6½-year-old ewes which conceived immediately produced respectively 1.69, 1.84, 2.52 and

2.82 lambs per head. Less evidence of association between age of ewe and lambing rate was obtained in Trial IV, in which the treated 3½- and 4½-year old ewes both produced 1.34, and the aged ewes 1.46 lambs per head. The observed effects are consistent with the view that at high levels of P.M.S. injection, the ovulation rate is raised to such a degree that it no longer limits fecundity, the size of the litters born being governed by the number of embryos the ewes are capable of carrying through pregnancy, this being greater for the older ewes. With smaller amounts of P.M.S., fewer eggs are ovulated, and even the younger ewes can carry most of the resulting embryos to full term.

(*b*) *Ewes which returned to service*: Comparing now the lambing performance of the treated and control ewes which returned to service, there is some indication of a delayed but reduced effect of P.M.S. on the proportion of multiple births. In Trial IV, 3 of the 28 control ewes (11 per cent) produced twins whereas 8 of the 21 treated animals (38 per cent) did so. This difference is significant ($P = 0.03$*). A similar but non-significant difference existed in Trial I (see Table VI). However, it should be noted that the 12 treated animals of Trials II and III which returned to service all produced single lambs. Further information is needed before definite conclusions can be drawn.

*Exact probability—see Fisher (1944).

(5) *Birth weights and lamb mortality*

Table VII presents data on the incidence of lamb mortality in each of the four trials.

TABLE VII. LAMB MORTALITY DATA

Trial	Group	Lambs Born	Deaths			Total	% Dead at 28 Days
			At Birth	1–7 Days	8–28 Days		
I	Control	99	3	1	2	6	6
	P.M.S.	141	9	14	3	26	18
II	P.M.S.	38	13	2	1	16	42
III	P.M.S.	83	20	3	—	23	28
IV	Control	143	17	11	—	28	20
	P.M.S.	165	11	18	6	35	21

Most of the deaths occurred either at birth or during the first week. In Trial I the death rate during the first 28 days was low amongst lambs from control ewes (6 per cent), but very high amongst those from the injected ewes (18 per cent). In Trial IV the mortality was heavy and almost equally severe amongst lambs from control and treated ewes (20 and 21 per cent deaths respectively); the high death rate was due in large part to exceptionally adverse weather conditions over the lambing period. Also, in this trial the ewes appeared to develop an iodine deficient condition, a

number of lambs being born very weak with grossly enlarged thyroid glands. However, the greatest mortality was experienced in Trials II and III, particularly in Trial II in which 42 per cent (16 of 38) of the lambs died before reaching 28 days of age.

From Table VIII it is clear that the death rate rose sharply as the number of young produced at birth increased.

TABLE VIII. THE EXTENT OF LAMB MORTALITY IN RELATION TO LITTER SIZE

Trial	Type of Birth	Lambs Born	Lambs Dead at 28 Days	Percentage Deaths	Lambs Reared per Ewe Lambing
I	Single	131	7	5	0.95
	Twin	76	14	18	1.63
	Triplet	21	6	29	2.14
	Quad.	12	5	42	2.33
II	Single	8	1	13	0.87
	Twin	4	1	25	1.50
	Triplet	18	7	39	1.83
	Quad.	8	7	88	0.50
III	Single	12	1	8	0.92
	Twin	16	3	19	1.63
	Triplet	33	8	24	2.27
	Quad.	16	5	31	2.75
IV	Single	177	24	14	0.86
	Twin	128	38	30	1.41
	Triplet	3	1	33	2.00
All Trials	Single	328	33	10	0.89
	Twin	224	56	25	1.50
	Triplet	75	22	29	2.12
	Quad.	36	17	47	2.11

Thus in Trial I, only 5 per cent of the lambs born as singles died during the first month, whereas, 18, 29 and 42 per cent of those born as twins, triplets and quadruplets respectively, did so. Similar trends are shown by the percentage mortality figures for the other trials. Clearly the greater mortality rate amongst lambs born to injected ewes is associated with the higher incidence of multiple births.

In spite of the extremely heavy mortality amongst lambs born in larger sized litters, the number of lambs reared per ewe tends to increase with litter size. This effect is clearly demonstrated in Table VIII, at the foot of which the overall results from all four trials are shown. No more lambs were reared by ewes with quadruplets than by ewes with triplets, but the numbers involved are too few to permit of any reliable conclusion being drawn. Even with the larger numbers of ewes with singles and twins the figures can be taken as providing only very approximate estimates of the numbers of lambs normally reared, for unusually heavy neo-natal mortality was experienced in these trials, particularly in Trial IV.

In Trials I, II and III, the lambs were weighed within 24 hours of birth, but in Trial IV birth weights were not recorded. In Table IX the mean birth weights of the lambs are classified according to sex and litter size. Male lambs were, in general, heavier than females, and there was a marked reduction in birth weight with increase in litter size, such that the mean birth weight of lambs born as quadruplets was only approximately half that of those born as singles. The heavy mortality amongst triplets and quadruplets was undoubtedly due in part to their low birth weight. There was considerable individual variation in birth weights, these ranging from 7.0–16.0 lb. for singles, 4.0–10.9 lb. for twins, 3.0–9.3 lb. for triplets and 3.0–9.0 lb. for quadruplets. In the case of twins, triplets and quadruplets, the lambs which failed to survive tended to be the lighter ones. In the case of singles, the mean birth weight was similar for those that lived and those that died; a number of the heavier ones were born dead, apparently as a result of difficult parturitions.

TABLE IX. BIRTH WEIGHTS OF LAMBS

Trial	Type of Birth	Number		Mean Weight	
		Males	Females	Males (lb.)	Females (lb.)
I	Single	55	61	11.8	11.3
	Twin	34	38	7.1	7.2
	Triplet	5	16	4.8	5.3
	Quad.	2	10	5.5	4.6
II	Single	5	3	12.3	12.2
	Twin	3	1	8.5	8.4
	Triplet	5	4	6.1	6.6
	Quad.	5	3	6.6	6.0
III	Single	7	5	10.9	10.5
	Twin	6	8	9.0	8.0
	Triplet	15	12	7.6	6.7
	Quad.	10	6	5.8	5.3
Total (I, II & III)	Single	67	69	11.8	11.2
	Twin	43	47	7.5	7.3
	Triplet	25	32	6.7	6.3
	Quad.	17	19	6.0	5.1

(6) *Lambing percentages*

It is common practice to measure the reproductive efficiency of sheep in terms of lambing percentages. In Table X, two sets of percentages have been calculated: (i) from the numbers of lambs born and of ewes lambing, and (ii) from the numbers of lambs alive at 28 days of age and of ewes mated. The overall result of treatment is better portrayed by the second set of values since these take into account the level of fecundity of the ewes which lamb, the proportion of animals which fail to conceive and the proportion of deaths amongst both ewes and lambs. The first set

of figures provides a useful measure of the special effect of P.M.S. treatment in inducing multiple births.

The percentages calculated from the numbers of lambs born and of ewes lambing [(i)], clearly show that P.M.S. treatment resulted in markedly increased fecundity. In Trial I, with the higher dose of P.M.S., the lambing percentage was 58 per cent greater for treated than for control animals; in Trial IV, with the smaller quantity of P.M.S., the difference amounted to 21 per cent. In both Trials II and III, in which the higher dose was used, lambing percentages of over 200 per cent were obtained.

The percentages based on the ewes mated and the lambs alive at 28 days of age [(ii)] show similar effects, although the differences between values for treated and control groups are considerably reduced. Nevertheless, in Trials I and IV, the final lambing percentages of the treated ewes were 32 and 12 per cent higher respectively than were those of the control animals. The treated ewes of Trial III had the highly satisfactory percentage of 160, but those of Trial II, only 110. In the latter trial only 11 of the 20 animals conceived to first service and lamb mortality was exceedingly heavy.

TABLE X. LAMBING PERCENTAGE DATA

Trial	Group	Ewes		Lambs		Lambing Percentage	
		Lambing	Mated	Born	Alive at 28 days	Lambs born per 100 ewes lambing	Lambs alive at 28 days per 100 ewes mated
I	Control	93	103	99	93	106	90
	P.M.S.	86	94	141	115	164	122
II	Treated	18	20	38	22	211	110
III	Treated	36	37	82	59	228	160
IV	Control	122	132	143	115	117	87
	P.M.S.	120	131	165	130	138	99

DISCUSSION

From a number of viewpoints the results obtained must be considered highly satisfactory. They clearly demonstrate that the treatment of ewes with P.M.S. on either the twelfth or thirteenth day of the oestrous cycle leads to a marked increase in the incidence of multiple births and to a substantially improved lambing percentage. They suggest that P.M.S. treatment does not make the ewes any more difficult to get in lamb, nor result in a greater proportion failing to become pregnant eventually. They indicate also that the extent of the increase in the numbers of lambs born is in large measure controllable; it is a matter of judicious choice

of dosage levels. If high doses of P.M.S. (e.g., 1,000 i.u.) are employed with fully mature Romney ewes (5½ or more years of age), the ewes that lamb may be expected to produce approximately two lambs per head and a few of them may have litters of four, five and even six lambs. If lower dose levels (400–500 i.u.) are used, such undesirably large litters are avoided, but the increase in the number of lambs born is less spectacular. A point that has received particular emphasis as a result of the present trials, is the need to carefully check the potency of the gonadotrophic material used.

In endeavouring to assess the possible usefulness of the P.M.S. technique in commercial practice, a number of factors have to be carefully considered. The first is the cost of the material. Commercially prepared dried P.M.S. costs in New Zealand approximately 7/6d. per 1,000 i.u. On the basis of the results that were obtained in Trial 1, for every three ewes injected at this level one would expect to obtain one additional lamb, alive at the age of a month. The cost is considerable but not prohibitive in view of the present high value of fat lambs. However, if the technique of P.M.S. treatment were to become widely applied, serious consideration would naturally be given to the collection and use of raw serum.

Apart from the cost of gonadotrophic material, some further expense is entailed in the provision of vasectomised rams although low priced animals are quite suitable for this purpose.

An important factor is the amount of labour which is involved in carrying out the treatment. At present this probably constitutes the greatest deterrent to extensive use of the technique. Over the period during which the vasectomised rams are with the flock, it is necessary to muster the animals every second day in order to raddle the rams, to identify in some way those ewes tupped by them during the previous 48 hours, and to inject those ewes tupped either 12 or 13 days earlier. In our experience, we have found it convenient to draft from the main flock at intervals of 12 days those ewes marked by the vasectomised rams over the preceding 12 days. This smaller group of animals can then be kept nearby and brought into the yards every second day for injection purposes. The regular raddling of the rams is probably the most laborious part of the procedure. The need for this would be obviated if a harness satisfactory for the purpose were developed.

In practice the interval between injecting occasions may well be extended to 3 or even 4 days. Wallace (1953) has recently shown that good results can be achieved by injection on the fourteenth and possibly on the fifteenth days after oestrus as well as on the twelfth and thirteenth days. Naturally, the fewer the occasions upon which injections have to be made, the less laborious is the technique to apply.

A further important consideration is the effect of treatment upon the time of lambing. On many New Zealand fat lamb farms, the rams are put out to the ewes at the beginning of March or even earlier. Normally this time more or less coincides with the

beginning of the breeding season, and under such circumstances most ewes are actually mated by the rams at the first heat of the season. The injection technique does not permit of ewes being mated before their second oestrus—hence, if carried out, lambing is likely to be delayed by perhaps 2–3 weeks. Under certain circumstances this may prove a disadvantage as, for instance, where early lambing is practised with a view to marketing as many lambs as possible fat off their mothers before pasture growth slows and its quality deteriorates with the advent of dry summer conditions. After P.M.S. treatment, very high percentages are to be expected and provision should be made for the finishing of the lambs. If such provision is made as, for example, by growing a summer crop, then the later lambing should prove no disadvantage. It might, in fact, be definitely desirable from the point of view of providing adequate feed for the increased number of stock during the early spring period.

In using the P.M.S. technique upon a commercial basis it would not be advisable to attempt to inject the whole flock. It would be best to concentrate upon those ewes which come into oestrus reasonably early in the season. In this way, most of the ewes could be treated over a comparatively short period, and the labour requirement considerably reduced. Moreover, an excess of late lambs would be avoided.

A further question is that of the age of the ewes to be injected. The results obtained indicate that the lambing rate following P.M.S. treatment is greater in older ewes, but a few undesirably large litters seem likely. For this reason, best results may in practice be obtained by treatment of $3\frac{1}{2}$- and $4\frac{1}{2}$-year-old ewes. It has been suggested that, due to embryonic mortality, these younger animals are rarely capable of carrying more than three lambs to full term. Further work is needed to confirm this hypothesis, and also to ascertain the effect of treatment upon the lifetime productivity of such animals. This latter aspect is of lesser importance in the case of $5\frac{1}{2}$- and $6\frac{1}{2}$-year-old ewes which would normally be killed after being bred for either one or two seasons.

Perhaps the most obvious place for the injection technique in farming practice at present would be in stud flocks and in the smaller fat lamb flocks. Stud flocks are suggested as suitable, for with them the cost of the material would be small in relation to the value of the additional lambs produced. Moreover, on properties carrying stud flocks, vasectomised rams are usually kept and tupping dates are often recorded. Also, greater shepherding attention is normally provided at lambing time. This is also true in the case of the smaller fat lamb flocks. Results obtained from P.M.S. treatment will largely depend upon whether lamb mortality can be kept within reasonable proportions.

In certain districts of New Zealand the disease of "facial eczema" constitutes a major hazard for fat lamb producers each autumn. It frequently happens that before and during the tupping period breeding ewes have to be heavily concentrated upon small

areas in order to prevent them from consuming toxic pasture. Flocks confined in this way are necessarily subject to a very low plane of nutrition, and for this reason can be expected to produce only a small proportion of twins at lambing. Under such circumstances, the P.M.S. injection technique should prove particularly useful. The ewes are usually kept in small paddocks near the sheep yards, the operations of drafting and injecting the ewes could be conveniently performed, and very worthwhile increases in lambing percentage should result from treatment.

In conclusion it may be said that in the future farmers probably will and probably should continue to rely, as they have in the past, upon the practice of flushing as the most ready method of securing good lambing percentages. However, feed for flushing is sometimes not available. On other occasions, although available, it either may not be used with safety, or may be very profitably employed for other purposes. Under such circumstances, a programme of P.M.S. treatment merits serious consideration as an alternative method.

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EXPERIMENTS IN CONTROL OF PEACH BROWN-ROT (*Sclerotinia fructicola* (Wint) Rehm.)

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Summary

Blossom-blight was reduced from 15 per cent to 2.1–3.5 per cent with applications of Phygon XL or Thirospray at 1 lb./100 gal. every three or four days during flowering. Spraying programmes which continued until within a few days of picking reduced fruit infection both at harvest and in storage. Flit-406 at 1 lb./100 gal. gave best control of fruit rot.

Introduction

Brown-rot of peaches in New Zealand is caused by the fungus *Sclerotinia fructicola* (Wint) Rehm. This organism affects fruit in all parts of New Zealand, particularly in the major peach-growing districts of Hawke's Bay and Auckland, and to a lesser extent in Central Otago. The fungus attacks flowers, causing blossom-blight, and fruit, where infection produces the characteristic soft, brown rot. The name *S. fructicola,* as applied to the common North American species replaces *S. cinerea,* the name used by Cunningham[1] for the species in New Zealand. *S. laxa* and *S. fructigena,* the two most important related species, do not appear to occur in New Zealand.

Commencement of apothecial production from mummified fruits in the soil beneath trees coincides with the blossoming of earlier-flowering peach varieties. In Auckland this is towards the end of August. Apothecia continue to appear for about a month. During this time old infected fruits still on trees, and twigs infected the previous season, do not produce conidia freely. Thus it seems that ascospores are responsible for the initial infection of flowers. However, once blossom-blight has begun, conidia are rapidly produced on dead flowers and are probably mainly responsible for spread of infection. From flowers, the fungus invades adjoining twigs and produces conidia at surfaces of lesions.

Young peach fruits are seldom attacked unless damaged. As harvesting approaches they are infected more often even though undamaged. In the last fortnight of ripening, and especially when fully ripe, peaches are easily infected under humid weather conditions. Spread of the disease through a crop can be rapid.

Two general methods of dealing with brown-rot are possible. The first, a programme of orchard sanitation, requires destruction of all rotten fruit and pruning out of infected twigs. If this work were carried out thoroughly, the fungus could possibly be eliminated from an orchard. However, trees are always liable to infection from neighbouring properties and for this reason spraying is necessary.

This paper deals with spraying experiments in three districts: Auckland, Hastings and Gisborne.

Materials and Methods

In the experiments described, spray application was by hand lance. Concentrations are given as the amount of chemical required for 100 gal. of mixture. The following materials were used:

Bordeaux mixture, x/y/100: copper sulphate (x lb.) plus hydrated lime (y lb.) in 100 gal.
Colsul 40: paste containing 40 per cent colloidal sulphur.
Crag 341 C: liquid containing 54 per cent glyoxalidine complex.
DDD–25: wettable powder containing 25 per cent T.D.E.
Flit–406: wettable powder containing 50 per cent captan.
Hexone: liquid containing 15 per cent tetraethyl pyrophosphate.
Lime sulphur: liquid containing 15 per cent polysulphides.
Phygon: wettable powder containing 85 per cent dichloronaphthoquinone.
Phygon XL: wettable powder containing 50 per cent dichloronaphthoquinone.
Santobrite: technical sodium pentachlorophenate.
Thirospray: wettable powder containing 50 per cent thiram.

The varieties used in all but one of the experiments were Paragon, which flowers in late August/early September and is picked in early February, or Golden Queen, which flowers in late September and is harvested in March.

To control leaf-curl, Bordeaux mixture, usually 10/8/100, was applied to all trees each season at bud movement. This is not shown in tables. For treatments from pink onwards, all experiments except those at Gisborne and at the private orchard in Auckland were laid out in randomised blocks of single tree replicates.

For records of blossom-blight late in flowering, at least 300 (200 in Gisborne) flowers taken at random from each tree were counted. Fruit records were made of the harvested crops only, and windfalls were not counted. Where storage trials were carried out, fruit was always held at prevailing summer temperatures in sheds. No experiments were done with cool storage.

Earlier Work

Prior to 1951, Mr. G. G. Taylor, formerly of this laboratory, was responsible for experiments on brown-rot control. The present paper deals mainly with work since 1951, when the author took over the problem, but Mr. Taylor's earlier experiments in Auckland are discussed briefly.

Programmes for seasons prior to 1951/52 are summarised, with results, in Tables I–VI. In the 1948/49 trials (Tables III and IV), Bordeaux mixture used during summer caused severe leaf and fruit damage, and intended January applications were omitted. Counts of infected flowers on Golden Queen in 1950 (Table VI) showed that the amount of blossom-blight did not differ between sprayed and unsprayed trees.

TABLE I. BROWN-ROT IN FRUIT OF TWO PEACH VARIETIES AFTER SPRAYING WITH DIFFERENT FUNGICIDES: AUCKLAND, 1947

	Percentage infected fruit			
	Phygon, ½ lb.	Phygon, 1 lb.	Lime sulphur, ½ gal. + Colsul 40, 2 lb.	No Spray
Paragon, sprayed once, 14 days before harvest	3.5	1.2	4.6	6.5
Golden Queen, sprayed twice, 17 and 7 days before harvest	16.9	11.1	50.2	62.0

TABLE II. EFFECT OF SEVERAL FUNGICIDES ON BROWN-ROT AMONGST FRUIT OF GOLDEN QUEEN: AUCKLAND, 1947/48 SEASON

Application Time	Programme				
	a	b	c	d	e
Late Pink and Early Petal Fall	Bordeaux 2/6/100	Lime sulphur, 1⅓ gal.	Phygon, 2 lb.	Phygon, 1 lb.	Unsprayed
15 days before harvest, and 4 days before harvest	Lime sulphur, ½ gal. + Colsul 40, 2 lb.	Lime sulphur, ½ gal. + Colsul 40, 2 lb.	„	„	„
Percentage infected fruit at harvest	40.0	21.3	11.4	12.7	52.0

TABLE III. EFFECT OF SEVERAL FUNGICIDES ON BROWN-ROT AMONGST FRUIT OF PARAGON: AUCKLAND, 1948/49 SEASON

Application Time	Programme				
	a	b	c	d	e
Pink	Bordeaux, 2/4/100	Bordeaux, 2/4/100	Phygon, 2 lb.	Phygon, 1 lb.	Unsprayed
Early November and early December	Bordeaux, 2/4/100 + Colsul 40, 2 lb.	Lime sulphur, ½ gal. + Colsul 40 2 lb.	„	„	„
Early January	No Spray	„	„	„	„
Percentage infected fruit at harvest	21.9	22.6	9.2	25.0	38.1

TABLE IV. EFFECT OF SEVERAL FUNGICIDES ON BROWN-ROT AMONGST FRUIT OF GOLDEN QUEEN: AUCKLAND, 1948/49 SEASON

Application Time	Programme a	b	c	d	e
Pink	Bordeaux, 2/4/100	Bordeaux, 2/4/100	Phygon, 2 lb.	Phygon, 1 lb.	Unsprayed
Early November and early December	Bordeaux, 2/4/100 + Colsul, 40, 2 lb.	Lime sulphur, ½ gal. + Colsul 40, 2 lb.	"	"	"
Early January	No spray	"	"	"	"
Early February	Bordeaux, 3/3/100	Bordeaux, 3/3/100	"	"	"
Percentage infected fruit at harvest	56.0	49.6	6.5	9.2	92.4

TABLE V. EFFECT OF SEVERAL FUNGICIDES ON BROWN-ROT AMONGST FRUIT OF PARAGON: AUCKLAND, 1950/51 SEASON

Application Time	Programme a	b	c	d	e
Pink and Petal Fall	Crag 341 C, 1 pt. + Hydrated Lime, 1 lb.	Crag 341 C, 2 pt. + Hydrated Lime, 1 lb.	Phygon, 1 lb.	Lime sulphur, 1⅓ gal.	Unsprayed
4 weeks before harvest and 12 days before harvest	"	"	Phygon XL, 1 lb.	Lime sulphur, ½ gal + Colsul 40, 2 lb.	"
Percentage infected fruit at harvest	31.8	30.4	10.1	18.7	59.4
Mean equivalent angle. Difference for significance at P 0.05 = 8.4	36.1	34.7	20.1	25.8	50.7

Recent Work

During the seasons 1951/52 and 1952/53, programmes of frequent applications, including blossom sprays, have been used, and several of the newer fungicides have been tested.

A. 1951/52 season, Auckland: On Golden Queen trees an experiment was run to find the value of eradicant spraying while trees were dormant and also to compare several fungicides during the remainder of the season. Tables VII and VIII show treatments given

TABLE VI. EFFECT OF SEVERAL FUNGICIDES ON BROWN-ROT AMONGST FRUIT OF GOLDEN QUEEN: AUCKLAND, 1950/51 SEASON

Application Time	Programme						
	a	b	c	d	e	f	g
Pink and Petal Fall	Crag 341 C, 1 pt. + Hydrated lime, 1 lb.	Crag 341 C, 2 pt. + Hydrated lime, 1 lb.	Phygon, 1 lb.	Lime sulphur, 1⅓ gal.	No spray	No spray	Unsprayed
15 days before harvest and 5 days before harvest	"	"	Phygon XL, 1 lb.	Lime sulphur, ½ gal. + Colsul 40, 2 lb.	Flit-406, 4 lb.	Flit-406, 2 lb.	"
Percentage infected fruit at harvest	41.9	47.1	14.6	28.0	17.7	18.3	82.6
Mean equivalent angle. Difference for significance at P 0.05 = 6.6	40.9	43.7	21.8	28.3	25.6	26.8	65.0

before flowering and those applied as fruit developed. No differences in blossom-blight or in amount of brown-rot in the fruit crop were significant. The only spray damage on fruit was skin discoloration caused by Phygon XL, which also delayed ripening.

TABLE VII. EXTENT OF BLOSSOM-BLIGHT AFTER APPLICATIONS OF VARIOUS FUNGICIDES TO GOLDEN QUEEN: AUCKLAND, 1951 (5 TREES PER TREATMENT)

Application Time	Programme					
Dormant	Santobrite, 4 lb.			No Spray		
Pink	Phygon XL, 1 lb.	Thirospray, 2 lb.	No Spray	Phygon XL, 1 lb.	Thirospray, 2 lb.	No Spray
Percentage infected flowers	17.4	22.2	20.6	15.3	23.3	30.2

TABLE VIII. EFFECT OF THREE FUNGICIDES ON AMOUNT OF FRUIT INFECTION ON GOLDEN QUEEN: AUCKLAND, 1951

Times of Application	Spray			
	a	b	c	d
On 31 October (4 weeks after Petal Fall), 27 November, 31 January, 21 February (11 days before harvest)	Phygon XL, ½ lb.	Thirospray, 1 lb.	Colsul 40, 2 lb.	Unsprayed
Number of trees	8	8	8	6
Percentage infected fruit at harvest	23.4	25.1	23.4	31.3

B. 1952/53 season, Auckland: Paragon trees were used in an experiment to compare effectiveness of four materials applied frequently. Programmes are given in Table IX. Since the amount of blossom infection was low on all trees, counts were not made. Flowers sprayed with Flit-406 were completely free of damage. With Phygon XL, blossoms did not appear as healthy as those not sprayed, but there was no burning. Thirospray caused some scorching of petal tips. Severe scorching and flower drop followed lime sulphur applications, and for this reason Phygon XL was substituted during the second part of the flowering period.

Results of brown-rot counts on fruit are set out in Table IX. While all treatments significantly reduced infection, Phygon XL and Flit-406 were best. However, fruits sprayed with Phygon XL developed olive-coloured skin blemishes and were several days later than other peaches in ripening. Sulphur sprays left an unpleasant heavy deposit on peaches, while Flit-406 and Thirospray did not affect the appearance of fruit.

TABLE IX. FRUIT INFECTION AT HARVEST AND IN STORAGE AFTER APPLICATIONS OF DIFFERENT FUNGICIDES TO PARAGON: AUCKLAND, 1952/53 SEASON. (8 TREES PER TREATMENT)

Application Time	Programme				
	a	b	c	d	e
Pink (22 August)	Phygon XL, 2 lb.	Flit-406, 2 lb.	Thirospray, 2 lb.	Lime sulphur, 1⅓ gal.	Unsprayed
Flowering (25, 29 August, 1, 4, 9, 15 September)	Phygon XL, 1 lb.	Flit-406, 1 lb.	Thirospray, 1 lb.	Lime sulphur, 1 gal. until 1 Sept. then Phygon XL, 1 lb.	"
Petal Fall (22 September)	"	"	"	Phygon XL, 1 lb.	"
20 October, 17 November, 15, 26, 30 January (3 days before harvest)	"	"	"	Lime sulphur, ½ gal + Colsul 40, 2 lb.	"
Percentage infected fruit at harvest	3.0	3.7	8.3	8.9	22.1
Mean equivalent angle. (Difference for significance at P 0.05 = 4.95)	9.92	11.08	16.73	17.38	28.06
Number (x) of days in storage to allow 50% of fruits to become infected	15	13	10	10	7
Log x. (Difference for significance at P 0.05 = 0.024)	1.1780	1.1046	1.0184	1.0022	0.8390

From each tree one tray containing 22 to 39 sound peaches in a single layer was stored and at intervals of two or three days counts were made of fruits which had become half rotten. This degree of decay was used since it allowed accurate counting without handling of fruit. By probit analysis the times taken for 50 per cent of fruits to become half rotten were estimated and these are given in Table IX. All materials prolonged the period of freedom from rot, but on fruit sprayed with Phygon XL skin blemishing became even more pronounced than at picking. Flit-406 had no effect on appearance or flavour of peaches and must, therefore, be considered to have been the best treatment.

On Golden Queen trees an experiment was laid down mainly to investigate the importance of spraying through the flowering period and during the last stages of fruit ripening. Programmes and results are given in Table X. Spraying at pink and during flowering reduced the amount of blossom-blight whereas applications at pink alone gave no reduction of infection. Although the results demonstrate a tendency towards increased fruit protection by spray applications close to harvest, wide variation between trees under the same programme made the error so large that only with Treatment (a) was the mean percentage of infection significantly lower than on unsprayed trees.

The addition of DDD-25 and Hexone in Treatment (a) was intended to prevent insect damage by leaf-roller caterpillars, in the case of the former, and thrips. There was, however, a negligible incidence of leaf-roller in the orchard, and the thrips, which invariably appear in large numbers on ripening fruit, soon reinfested Hexone-sprayed trees from those adjacent. It remains to be determined whether insect damage is a significant factor in fruit infection.

One tray of sound fruit from each tree was held for seven days and the number of half-rotten fruit counted. Table X shows that Treatments (a), (b) and (d) gave better control of rot in storage than treatments which ceased two weeks before picking. Treatment (d), with late sulphur sprays, left a heavy deposit on fruit, but other treatments gave little visible residue.

C. *Blossom-spraying trial, Auckland 1952*: An experiment was carried out in a commercial orchard to gain more information on the effect of spraying during flowering. Seven trees at the end of each of four rows, one of Paragon, one of A1, and two of Stark were sprayed. Applications commenced at pink (22 August for Paragon, 4 September for other varieties), and continued until petal fall, spraying dates being as follows: 25 August, 1, 4, 8, 11, 15, 18, 22 and 26 September. Until 1 September, lime sulphur 1 gal. was used, but as this scorched flowers, Phygon XL 1 lb., was applied for the remainder of the period.

All trees were grown high and most flowers were too far from the ground to be examined. Records were made, therefore, six weeks after petal fall when all peaches on the seven sprayed trees of each row and on the adjoining seven were counted. Figures from these counts are as follows:

Variety	Mean Number Sprayed	Mean Number Unsprayed
Paragon	785	475
Stark (1st row)	569	233
Stark (2nd row)	358	122
A1	305	109

In every row there were significantly more peaches, at P 0.01, on sprayed than on unsprayed trees.

TABLE X. EFFECT ON BLOSSOM-BLIGHT AND ON FRUIT INFECTION AT HARVEST AND IN STORAGE OF VARIATION IN BOTH FUNGICIDES USED AND APPLICATION TIMES ON GOLDEN QUEEN: AUCKLAND, 1952/53 SEASON (6 TREES PER TREATMENT)

Application Time	Programme					
	a	b	c	d	e	f
Pink (12 September)	Thirospray, 2 lb.	Thirospray, 2 lb.	Thirospray, 2 lb.	Lime sulphur, 1⅓ gal.	Lime sulphur, 1⅓ gal.	Unsprayed
Flowering (15, 18, 22, 26, 30 September, 3 October)	Thirospray, 1 lb.	Thirospray, 1 lb.	No Spray	Phygon XL, 1 lb.	No Spray	"
Petal Fall (10 October)	"	"	Thirospray, 1 lb.	Lime sulphur, 1 gal.	Lime sulphur, 1 gal.	"
10 November	"	"	"	Lime sulphur, ½ gal. + Colsul 40, 2 lb.	Lime sulphur, ½ gal. + Colsul 40, 2 lb.	"
9 December and 10 February	Thirospray, 1 lb. + DDD-25, 1 lb.	"	"	"	"	"
23 February	Thirospray, 1 lb.	"	"	"	"	"
2 March and 6 March (3 days before harvest)	Flit-406, 1 lb. + Hexone, 1 pt.	Flit-406, 1 lb.	No Spray	"	No Spray	"
Percentage infected flowers on 30 September	3.5	4.3	20.9	2.1	17.2	15.0
Mean equivalent angle. (Difference for significance at P 0.05 = 5.45)	9.73	11.35	26.61	7.21	23.77	22.16
Percentage infected fruit at harvest	12.7	16.4	44.3	24.2	42.7	52.4
Mean equivalent angle. (Difference for significance at P 0.05 = 22.66)	20.91	23.87	41.74	29.45	40.78	46.35
Percentage infection in fruit stored for 7 days	7.6	12.9	42.0	12.0	33.1	40.7
Treatments significantly poorer at P 0.05	c, e, f	c, e, f	None	c, e, f	None	None

D. 1951/52 season, Hastings: Three fungicides were compared on Golden Queen trees. Table XI summarises the four programmes. Since the amount of blossom-blight on unsprayed trees was negligible, counts were not made. Results of fruit counts show that all materials lowered the amount of infection, but no distinction could be made between the three sprays.

From each treatment, four trays of clean fruit picked five weeks after the final spray were stored. Table XI shows that after ten days there was not significantly less infection with Flit-406 and Thirospray than amongst unsprayed fruit, while peaches sprayed with sulphur had significantly more rot at P 0.05, than untreated.

E. 1952/53 season, Hastings: Golden Queen trees were used to test the effect on the amount of brown-rot at harvest of including blossom sprays in a programme. In Table XII, programmes and results are given. Flower infection was light. Both fungicide treatments reduced brown-rot of fruit. Although infection on trees which had no applications during flowering was greater than on those which had, the difference was not significant.

TABLE XI. FRUIT INFECTION AT HARVEST AND IN STORAGE AFTER APPLICATIONS OF DIFFERENT FUNGICIDES TO GOLDEN QUEEN: HASTINGS, 1951/52 SEASON. (5 TREES PER TREATMENT)

Application Time	Programme			
	a	b	c	d
Pink (5 September)	Flit-406, 2 lb.	Thirospray, 2 lb.	Lime sulphur, 1⅓ gal.	Unsprayed
Petal Fall (23 September)	„	„	Lime sulphur, 2/3 gal.	„
8 October, 6 November, 26 November, 26 January, 19 February (3–5 weeks before harvest)	Flit-406, 1 lb.	Thirospray, 1 lb.	Lime sulphur, ½ gal. + Colsul 40, 2 lb.	„
Percentage infected fruit at harvest	2.3	2.5	5.5	18.5
Mean equivalent angle. (Difference for significance at P 0.05 = 8.55)	6.24	7.89	12.86	25.14
Percentage infection in fruit picked 24 March and stored for 10 days	0.0	2.3	18.0	4.4
Mean equivalent angle. (Difference for significance at P 0.05 = 15.0)	0.0	6.0	23.5	8.2

From each tree, one tray of clean fruit picked eight days after spraying was stored, and counts were made every three or four days until all peaches had become infected. The periods after harvest required to allow 50 per cent of peaches to become infected were calculated. Table XII shows that both programmes improved keeping time by a significant amount compared with the controls. There was no difference between programmes.

From the same pick, four trays of fruit from each programme were sent by rail from Hastings to Auckland. In each tray a single layer of peaches was packed between layers of wood wool. Table XII shows percentages of infected fruit four days after consignment. Again sprayed fruit was cleaner than unsprayed, with least infection amongst peaches from trees sprayed during flowering.

TABLE XII. EFFECT OF BLOSSOM SPRAYS ON AMOUNTS OF INFECTION AMONGST FLOWERS, FRUIT AT HARVEST AND FRUIT IN STORAGE FROM GOLDEN QUEEN: HASTINGS, 1952/53 SEASON. (8 TREES PER TREATMENT)

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Application Time	Programme		
	a	b	c
Pink (11 September)	Thirospray, 2 lb.	Thirospray, 2 lb.	Unsprayed
Flowering (15, 19, 22, 26 September)	No Spray	Thirospray, 1 lb.	"
Petal Fall (30 September)	Thirospray, 2 lb.	"	"
22 October, 28 November, 3 February, 23 February, 4 March, 10 March (1–16 days before harvest)	Thirospray, 1 lb.	"	"
Percentage infected flowers	0–2	0	0–3
Percentage infection in ripe fruit	5.6	1.0	17.7
Mean equivalent angle. (Difference for significance at P 0.05 = 8.16)	13.74	5.71	24.88
Number of days in storage of fruit picked 8 days after spraying to allow 50% of peaches to become infected. (Difference for significance at P 0.05 = 5)	17	20	10
Percentage infection in fruit opened after transport to Auckland	12	5	46

TABLE XIII. EFFECT OF DIFFERENT FUNGICIDES ON BLOSSOM-BLIGHT AND FRUIT INFECTION ON GOLDEN QUEEN: GISBORNE, 1952/53 SEASON. (8 TREES PER TREATMENT)

Application Time	Programme		
	a	b	c
Pink (12 September)	Lime sulphur, 1¾ gal.	Phygon XL, 1 lb.	Thirospray, 2 lb.
Flowering (15, 19 22, 26 28 September) Petal fall (1 October, 14 October, 19 December, 7 February)	Lime sulphur, ½ gal. + Colsul, 40 2 lb.	"	"
16 February, 23 February, 1 March (1 day before harvest)	"	Phygon XL, ½ lb.	Thirospray, 1 lb.
Percentage infected flowers	16.5	11.4	10.7
Mean equivalent angle. (Difference for significance at P 0.05 = 4.25)	23.01	19.40	18.42
Percentage infected fruit at harvest	6.3	2.6	6.5
Mean equivalent angle. (Difference for significance at P 0.05 = 4.66)	11.86	8.72	13.98

F. 1952/53 season, Gisborne: On Golden Queen trees full programmes were used to compare three materials. Each programme was applied to two four-tree replicates. Programmes and results are set out in Table XIII. There was least blossom-blight with Thirospray and lowest fruit infection followed use of Phygon XL. Thirospray caused an unhealthy appearance of foliage and some fruit drop. With Phygon XL, fruit suffered skin blemishing similar to that found in Auckland.

Discussion

In experiments at Auckland before the 1951/52 season, spraying programmes consisted of up to five applications and some control of fruit infection was obtained. For the 1951/52 season a schedule recommended for New Zealand stone fruits[2] was followed with minor deviations. This calls for applications at late pink and petal fall, with four subsequent sprays up to approximately two weeks before harvest. On Golden Queen in Auckland this programme caused no reduction in blossom-blight and no control of fruit infection. The amount of blossom-blight, which probably depends in the early stages largely on infection from asci on the ground

and not on production of conidia at twig lesions, was not affected by spraying trees while dormant with Santobrite.

Prevention of blossom-blight was investigated in the 1952/53 season. In Auckland, spraying every three or four days throughout blossoming on Golden Queen reduced flower infection considerably. By reducing the load of conidia in an orchard with control of blossom-blight, fruit infection at harvest could be expected to be lightened. In the 1952/53 Hastings trial, where however there was little blossom-blight even on unsprayed trees, trees sprayed during flowering had less rot on fruit than those unsprayed at flowering but given the same subsequent treatment, though the difference was not significant. In the commercial orchard in Auckland the number of peaches set on sprayed trees was greater than on unsprayed, a fact taken to show that blossom-blight had been less severe on the former. The heavy programmes used during flowering are expensive but it may be possible to obtain the same result with fewer sprays.

As infection was regularly heavy on Golden Queen in Auckland and unsprayed trees scattered through the blocks were a source of infection to surrounding trees, development of fruit-rot on a sprayed tree was regarded as being independent of its blossom infection. For this reason, in considering the amount of brown-rot at harvest, only sprays after petal fall were taken into account. Golden Queen trees having applications seven days, and again three days, before picking had less infection than those not sprayed later than fourteen days prior to harvest, but the difference was not significant.

An assessment may be made of the relative values of fungicides used in the experiments. In control of blossom-blight, Phygon XL 1 lb., and Thirospray 1 lb. were equally effective, and rather better than the combination of lime sulphur ½ gal. with Colsul 40 2 lb. No data are available on the merits of Flit-406 in blossom sprays, but it did not cause damage, whereas lime sulphur at one gallon scorced flowers severely. Against fruit infection, Phygon, Phygon XL and Flit-406, all at 1 lb., gave best protection, but both Phygon and Phygon XL delayed ripening and caused olive-coloured skin blemishes. Thirospray 1 lb. and lime sulphur ½ gal, plus Colsul 40 2 lb. were not as good. Late use of sulphur combinations left heavy deposits, and at Gisborne, though not elsewhere, Thirospray caused leaf damage and fruit drop. In the small amount of work done with it, Crag 341 C did not show promise.

Serious infection of fruit can occur after harvest. When records were kept of brown-rot amongst peaches stored at pre-

vailing summer temperatures, it was found that fruit sprayed within a week of picking remained cleaner than that left unsprayed, or sprayed not later than two weeks before harvest. In the 1952 Hastings crop, a final application of fungicides one month before harvest did not improve the keeping quality of fruit. Of the fungicides used shortly before picking, Flit-406 1 lb., without causing spray damage, kept peaches free of rot longer than other materials.

Acknowledgments

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BLAST OF STONE-FRUIT IN NEW ZEALAND

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(Received for publication, 27 October 1953)

Summary

Blast of stone-fruit, a serious disease of all species of stone-fruit, occurs throughout New Zealand.

The disease infects trees of all ages, but is particularly serious on trees less than six years old.

Infection occurs on trunks, branches, twigs, leaves and fruits. Trees or branches are killed when girdled by cankers. Twigs are rapidly killed back from the tips and isolated lesions may occur wherever the causal organism gains entry through the cuticle. Leaf lesions usually occur as circular spots which are often watersoaked in appearance. Symptoms on fruits vary from superficial scab-like spots to large necrotic lesions which penetrate the flesh.

The morphology and cultural and physiological reactions of the causal organism agree with those given for *Pseudomonas syringae* van Hall by Bryan (1928).

Introduction

Isolations on nutrient agar from lesions on twigs of apricot (*Prunus armeniaca* L.), peach (*Prunus persica* Sieb. and Zucc.) and plum (*Prunus spp.*) received from Alexandra and Christchurch in September and October 1951, consistently yielded large numbers of similar

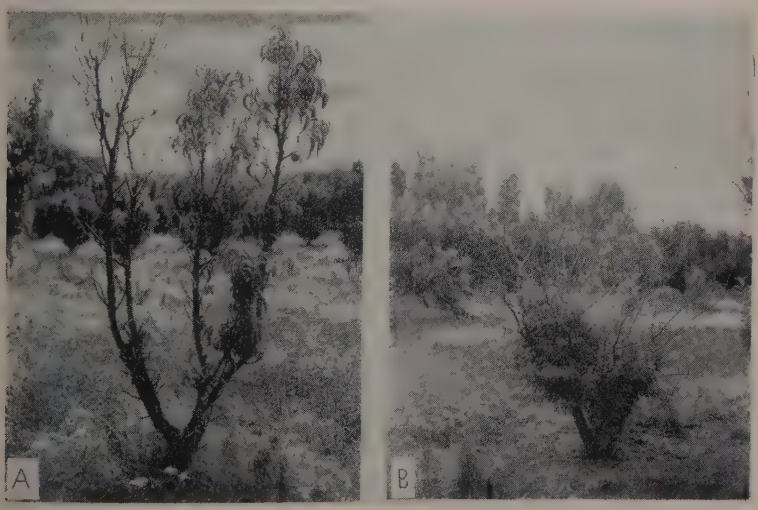

Fig. 1.—Effect of blast of stone-fruit on trees.
A. Peach; two leaders girdled in spring.
Photo: S. A. Rumsey
B. Apricot; strong growth from base of branches killed by *Ps. syringae*.
Photo: E. E. Chamberlain

bacterial colonies. An inspection in the Alexandra and Roxburgh districts in November 1951 revealed that a large proportion of the young stone-fruit trees in these districts was affected with a disease causing death of branches and trees (Fig. 1 A and B). Lesions on leaves and fruit from these trees contained numerous bacteria similar

Fig. 2.—Twig and branch symptoms of stone-fruit blast.

A. Apricot; internal staining of bark.

Photo: S. A. Rumsey

B. Peach; external and internal symptoms of lesions. Note gum drop and dead twig.

Photo: J. W. Endt

C. Plum; infection of buds.

Photo: S. A. Rumsey

D. Cherry; typical depressed lesion and gum.

Photo: J. W. Endt

to those isolated previously from twigs. Subsequent investigations have shown that the organism causing this disease is *Pseudomonas syringae* van Hall, which also causes Lilac-blight and blast of citrus, avocados, roses and many other hosts (Gussow, 1908; Coit, 1916; Smith, 1926; Rosen, 1935).

This disease is serious in New Zealand stone-fruit orchards and in nurseries. It attacks all stone-fruits, viz. almond, apricot, cherry, nectarine, peach, plum, and while it infects trees of all ages, most damage occurs on trees up to 6 years old. Because of this, difficulty is being experienced in establishing new plantings. In some blocks of young trees over 90 per cent have been killed in one year.

The disease is not a new one, having been present in New Zealand for many years. It has been known under such descriptive names as "sour-sap", "die-back" and "gummosis."

Symptoms

1. *Branches*: The organism attacks and rapidly kills the bark, causing it to be stained internally a bright orange to dark brown colour, clearly distinguishable from the almost white healthy tissue (Fig. 2 A). Diseased bark when cut gives off a strong sour odour. Cankers extend more rapidly vertically than horizontally and may be up to 100 cm. long before the stem is girdled and the branch or tree killed. There is often a copious exudation of water-soluble gum from infected branches (Fig. 2 B and D). In the dormant period lesions are not obvious, but become conspicuous in spring when they remain as depressed areas while the surrounding tissue grows (Fig. 2 D). In spring, after lesions cease to increase in size, the healthy tissues surrounding them produce a callus which clearly defines the borders of cankers. Cankers then become dry, cracked, and dark brown to black in colour.

Branches girdled before bud movement are killed and are very conspicuous when the remainder of the tree breaks into leaf. There is often a prolific growth of healthy foliage below the canker (Fig. 1 B). If a branch is almost girdled by a canker before bud movement the foliage that forms is usually pale green to yellowish green in colour and leaves are slightly narrower than usual, with margins curled upwards, giving them a rolled appearance. In addition, shoots are shorter and foliage appears to be sparse and unthrifty (Fig. 3 A). Development of cankers below the crotch may cause apparently healthy trees in full leaf to wilt suddenly and die in early summer.

Although most stone-fruit blast bacteria in cankers die during summer, some remain alive but inactive until the following autumn. Pathogenic bacteria have been isolated from stem cankers every month of the year.

2. *Twigs*: Infection progresses rapidly in young twigs, often killing them back 30 cm. or more from the tips in 2–3 weeks. They have a characteristic sour smell, soon become brown to black in colour and often exude large drops of gum. Small lesions may also occur. On apricot and cherry these are oval or irregularly shaped depressed

Fig. 3.—Leaf and fruit spur infection symptoms of stone-fruit blast.

A. Peach; rolled leaves and leaf lesions.
Photo: J. W. Endt

B. Almond; young and old lesions.
Photo: J. W. Endt

C. Apricot: lesions in all stages.
Photo: S. A. Rumsey

D. Apricot; fruit spurs in different stages of infection.
Photo: J. W. Endt

areas, dark brown to black in colour (Fig. 2 D). On peach and nectarine they are light brown, depressed areas with dark brown margins often surrounded by reddish zones (Fig. 2 B). The bark under lesions is stained light reddish brown or dark brown and is sharply defined from healthy tissue. Frequently lesions are confined to the nodes, the internodes remaining healthy (Fig. 2 C). On some shoots all buds may be killed for a distance of 60 cm. or more.

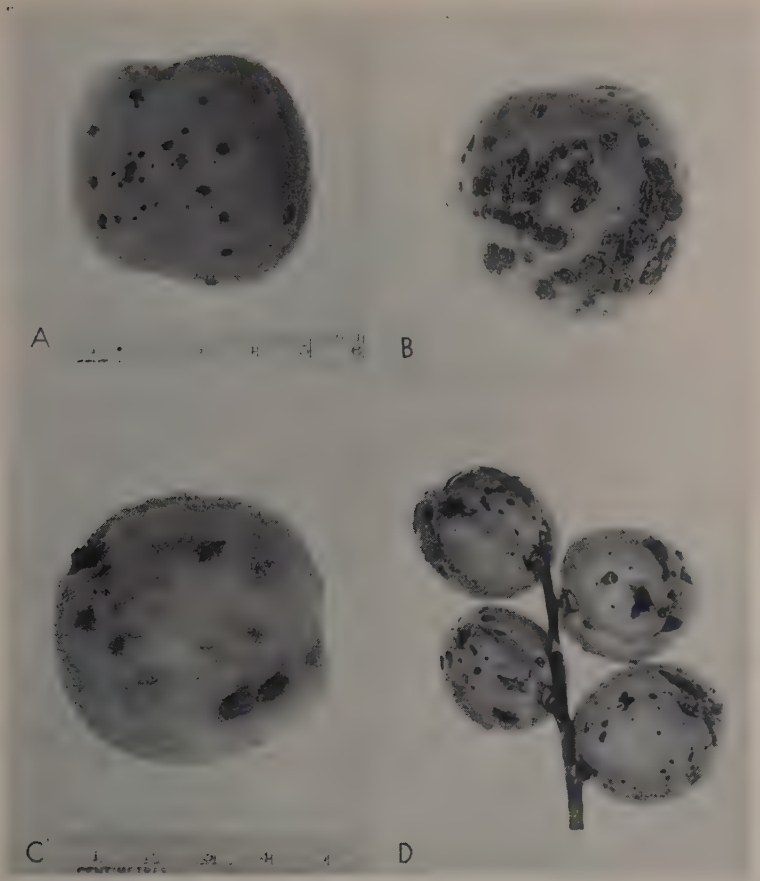

FIG. 4.—Infections of blast of stone-fruit on fruit.

A. Apricot; young lesions. *Photo: J. W. Endt*

B. Apricot; old lesions. *Photo: J. W. Endt*

C. Peach: severe infection. *Photo: S. A. Rumsey*

D. Nectarine; lesions on fruit and spur. *Photo: M. J. Dahlberg*

3. *Roots*: Pathogenic bacteria have been isolated from roots, but normally infection does not progress beyond the bud union. Infection in roots shows as brown stained, sour smelling phloem clearly distinguishable from healthy tissue.

4. *Leaves*: Symptoms of blast of stone-fruit on leaves can be confused in the field with Shothole (*Phyllosticta prunicola* Sacc.), Bacterial spot of plum and peach (*Xanthomonas pruni* (Erw. Smith)) and spray damage. Symptoms, which vary with species of host and age of leaf when infected, are presented in Table I.

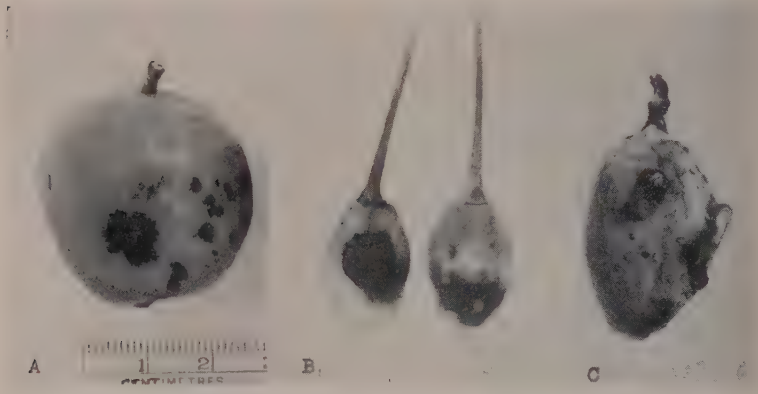

FIG. 5.—Infections of blast of stone-fruit on fruit.

A. Plum; typical lesions. *Photo: S. A. Rumsey*

B. Cherry; severe infection. *Photo: W. E. Barnard*

C. Almond; raised lesions and gum exudate. *Photo: S. A. Rumsey*

TABLE I. DESCRIPTION OF LESIONS CAUSED BY BLAST OF STONE-FRUIT ON LEAVES

	Apricot	Cherry	Almond, Plum, Peach, Nectarine
Early stage young leaves	Circular, 1-2 mm. diam., bright red with narrow yellow halo, becoming dark red or reddish purple.	Angular or circular, 1-2 mm. diam., dark green water-soaked, often with yellow halo.	Circular or angular, 1-3 mm. diam., olive green to light brown with water-soaked appearance. Lesions occur particularly at leaf tip and margin.
Early stage older leaves	Circular, 1 mm. diam., dark red to brown with definite yellow halo, becoming almost black with light brown centre.	Angular or circular, 1-2 mm. diam., dark green water-soaked, becoming brown.	Circular or angular, 1-3 mm. diam., reddish brown, more noticeable from lower surface, becoming dark red or dark brown. Lesions occur particularly at leaf tip and margin.
Final stage all leaves	Lesions become dry and brittle then fall from leaf leaving circular or irregular holes 2-5 mm. diam. (or more when lesions coalesce) and often removing the whole leaf tip and margin.		

5. *Fruit*: Symptoms of stone-fruit blast on fruit vary considerably with species of host and age of fruit when infected, as shown in Table II.

ISOLATION OF CAUSAL ORGANISM

The causal organism is readily grown on beef peptone media when isolated from woody stems in spring, or from young lesions in leaves, shoots and fruits. Isolation is difficult from woody stems in summer and autumn and from brittle "shothole" lesions of leaves. To test pathogenicity, many single colony isolations from stem, leaf and fruit lesions on all species of stone-fruit were needle inoculated into peach and plum seedlings in a glasshouse and into apricot, cherry, and peach trees in the field. Lesions typical of natural infections were formed in 6 days on glasshouse plants and were evident within 14 days in the field.

TABLE II. DESCRIPTIONS OF LESIONS CAUSED BY BLAST OF STONE-FRUIT ON FRUIT

	Apricot	Almond	Cherry	Nectarine	Peach	Plum[+]
Young lesions	Flat, superficial, mainly circular, dark red. 1-2 mm. diam. (Fig. 4A)	Raised, circular, scab-like, brown. 1-2 mm. diam.	Flat, irregular, dark brown to black. 2-3 mm. diam.	Flat superficial, circular, dark brown to black, often with red margin. 1-3 mm. diam.	Flat, superficial, circular, brown with red margin. 1-3 mm. diam.	Slightly raised, circular, olive green. 1-3 mm. diam.
Old lesions	Raised, scab-like, concentrically cracked, sometimes depressed in centre, light to dark brown. 3-6mm. diam. (Fig. 4B) Penetration seldom more than 2 mm.	Depressed, brown circular, 2-5 mm. diam. Gum frequently exuded. (Fig. 5C). Penetration 2-3 mm.	Depressed, irregular, black 5-15 mm. diam. (Fig. 5B) Penetration to stone.	Depressed, irregularly circular, black, 2-5 mm. diam., sometimes cracked. Gum frequently exuded. (Fig. 4D) Penetration 2-10 mm.	2 Types. A. surface lesions depressed, irregularly circular, dark brown or light brown centre, dark brown margin and dark red halo. 2-10 mm. diam. Gum frequently exuded. Penetration 2-10 mm. (Fig. 4C). Underlying tissue dark brown to black, sometimes spongy. B. internal lesions* dark brown to black spherical masses mainly near surface, sometimes spongy.	Depressed, irregular, sometimes cracked, dark brown to black centre with green water-soaked margin. 5-10 mm. diam. Gum frequently exuded (Fig. 5A). Penetration 2-5 mm.

* There are also brownish water-soaked spherical areas and brown-stained vascular strands, which contain large numbers of bacteria.

[+] These lesions can be confused with those of Bacterial spot of plum and peach (Xanthomonas pruni).

Identification of Causal Organism

Identification of the causal organism was determined according to morphology and cultural and physiological reactions suggested by the Society of American Bacteriologists in the "Manual of Methods for Pure Culture Study of Bacteria" (1946).

Morphology

The bacteria exist singly and in pairs as short rods with rounded ends. Single rods vary from 0.8μ to 2.2μ by 0.5μ to 0.7μ with an average of 1.4μ x 0.6μ. The rods are gram negative (Hucker stain). They are motile by one to several polar flagella (Gray and Leifson stains).

*Cultural Characteristics**

Beef Peptone Agar Colonies: Growth at 25° c. in 48 hr. moderate, circular, smooth, entire to undulate, convex later becoming flat, translucent, greyish white, no pigment formed in media.

Beef Peptone Agar Slope: Moderate, filiform, glistening, greyish white, slight odour, butyrous, medium unchanged.

Beef Peptone Broth: 7 days, no surface growth, slight clouding, no colour change, scanty white flocculent sediment, no odour: 21 days, moderate clouding, no surface growth.

Potato Slice: 7 days, growth moderate never abundant, moist, glistening, butyrous, filiform, buff coloured, potato unchanged; 50 days, no further change.

Nutrient Gelatin Stab: 10 per cent at 25° c. infundibuliform liquifaction in 24 hr., later becoming stratiform, 1–2 in. liquified in 10 days with white viscid sediment.

Plain Gelatin Stab: 10 per cent at 25° c. infundibuliform liquifaction in 16 hr., later becoming stratiform, complete in 10 days with white viscid sediment.

Purple Milk: Clearing in bands shows within 7 days, three zones being evident, viz. top 15-20 mm., translucent, iridescent red-blue; centre 15-20 mm. slightly cleared, reddish; bottom 8-10 mm. opaque, greyish white. In 14 days pH approx. 6.0, completely cleared, translucent, iridescent red-blue, with greyish white floccular sediment.

Nitrates: No nitrite production in nitrate broth or in beef peptone agar plus nitrate in 7 days.

Indole: Not formed.

Hydrogen Sulphide (Beef peptone agar plus sodium thiosulphate): No hydrogen sulphide in 21 days.

* Difco media used where applicable.

Starch (Beef peptone agar plus 0.2 per cent soluble starch): Rapid sometimes irregular spreading growth on fresh plates. No hydrolysis in 24 hr. or 30 days.*

M.R. Negative. *V.P.* Negative.

Oxygen requirement: Strict aerobe**

*Synthetic Carbohydrate Media****: 1 per cent. No gas in any medium. Acid in arabinose, xylose, glucose, fructose, galactose, mannose, sucrose, raffinose, glycerol, mannitol, sorbitol, in 21 days: Lactose and rhamnose, no acid in 28 days but acid in 50 days; Maltose, starch, inulin, dextrin, salicin, melezitose, no acid in 50 days.

Salt (Beef peptone broth plus NaCl): 7 days, growth in 2 per cent equal to check, moderate clouding, no pellicle. No growth above 6 per cent in 21 days. Sucrose 0.5 per cent included in above salt solutions caused strong clouding and pellicle formation but no change in growth range.

Additional Tests

Beef Peptone Agar plus 5 per cent Sucrose: Colonies circular, smooth, entire, hemispherical, white, opaque, have tendency to become translucent in centre after 7 days, no loss of viability in 7 days.

Beef Peptone Sucrose Agar Stroke: Abundant filiform, glistening, white at first; after 7 days middle of streak clears, becomes less viscid, frequently flows to base of slope as creamy white fluid which separates into a transparent fluid above a white opaque fluid.

Purple Lactose Agar Stab†: Good surface growth greyish white, no acid formation in 21 days.

Fluorescence: In Erikson's medium* a strong yellow green fluorescence observed under ultra violet light.

Pigment Production: No definite green pigment observed in 21 days in in any of the following medias:

(1) Erikson's medium;*

(2) Erikson's medium having glucose as carbon source and sodium ammonium phosphate 4.9 g./l. as nitrogen source;

(3) Erikson's medium as in (2) plus agar;

* Result of not less than 30 tests with cultures from 5 different sources. Each test consisted of two bacterial streaks on starch agar plates, one of which was wiped off before flooding with Lugol's solution.

** No growth in 14 days in beef-peptone sucrose-agar shake cultures covered with liquid paraffin, in beef-peptone agar shake cultures incubated in evacuated jar containing sodium pyrogallate or on beef-peptone slopes incubated in evacuated jar containing sodium pyrogallate.

*** Using brom-thymol-blue as indicator; media adjusted to pH 7.0 with normal NaOH; result of not less than 3 tubes of each of 4 cultures isolated from apricot, almond, cherry and peach.

† 2 per cent lactose, beef-peptone agar, brom-cresol-purple.

*Erikson, 1945	$K_2H.P.O_4$	1.0	g./l.	
	$MgSO_4.7H_2O$	0.5	g./l.	
	KCL	0.5	g./l.	
	$FeSO_4.7H_2O$	0.005	g./l.	
	Carbon source (Sodium fumarate)	10	g./l.	pH = 7.0
	Nitrogen source (Sodium nitrate)	2.0	g./l.	

(4) Beef liver infusion broth;

(5) Beef liver infusion broth plus glucose;

(6) Beef liver infusion broth plus sodium fumarate;

(7) Beef liver infusion agar;

(8) Beef peptone broth;

(9) Beef peptone broth plus 0.5 per cent sucrose;

(10) Beef peptone agar.

Factors Contributary to Development of the Disease

In New Zealand the organism causing blast of stone-fruit is most active in cold wet weather, especially in succulent growth. Infection is dependent on injuries such as those caused by wind, driving rain, hail, frost, insects, cultural operations or certain therapeutant sprays. The causal organism can also enter plants through leaf scars for a short period after leaf fall. In New Zealand leaf fall has been induced by transplanting young peach trees. Such trees when sprayed shortly after leaf fall with a culture of *Ps. syringae* were severely infected at more than 90 per cent of the leaf scars, whereas check trees, defoliated but not inoculated, and inoculated but not defoliated, remained healthy. Crosse (1951) using a closely related organism, *Pseudomonas mors-prunorum* (Wormald) on cherry spurs in England found that possible leaf-scar infection decreased from 98 per cent to 33 per cent in the first 8 days after leaf fall.

Conclusion

On the basis of morphology and physiology the New Zealand organism causing the stone-fruit disease is comparable with *Ps. syringae* and any differences between it and other similar organisms are small and can be attributed to strain variation. In symptoms, this organism agrees with those caused by *Pseudomonas mors-prunorum* (Wormald) and *Pseudomonas prunicola* (Wormald) on plum and peach (Wormald, 1930, 1931, 1942) and apricot (Crosse, 1953). Cultural characters do not agree with those of *Ps. mors-prunorum* since the organism described here does not produce acid in lactose, maltose or 2 per cent purple lactose agar in 28 days, nor does it lose viability on sucrose agar in 5-6 days, which characters were regarded by Wormald as important in distinguishing between *Ps. mors-prunorum* and *Ps. prunicola*. It does, however, agree reasonably well with the morphological and physiological characters of *Ps. prunicola* and *Pseudomonas cerasi* (Griffin, 1911), which are now considered to be synonyms of *Ps. syringae* (Wilson, 1931, 1936, 1940).

The common name "blast of stone-fruit" is suggested for this disease to conform with "blast" of citrus, avocados, roses and other plants also caused by *Ps. syringae*.

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DIFFERENTIAL EFFECT OF THERAPEUTANTS ON BACTERIAL-CANKER OF TOMATOES (*Corynebacterium michiganense* (Erw. Smith) Jensen) AND ON LATE-BLIGHT (*Phytophthora infestans* (Mont.) de Bary)

By D. W. Dye, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(Received for publication, 23 October 1953)

Summary

In the 1952-53 season dwarf tomato plants infected with bacterial-canker (*Corynebacterium michiganense*) were sprayed with Bordeaux mixture, Thirospray, Ascospray, Phygon XL and water.

Phygon XL markedly reduced infection of fruit by bacterial-canker; all other materials were ineffective.

Bordeaux sprays led to a considerable increase in bacterial-canker infection.

Late-blight (*Phytophthora infestans*) was efficiently controlled by Phygon XL and Bordeaux sprays and to a slightly lesser extent by Thirospray.

Introduction

In a previous paper Dye (1952) indicated that fruit infection of tomatoes by bacterial-canker (*Corynebacterium michiganense* (Erw. Smith) Jensen) could be reduced by spraying with Phygon XL, whereas copper sprays increased infection, and led to a decrease in yield. As Bordeaux mixture and other copper sprays are normally used for control of late-blight on tomatoes, trials were carried out in the 1952-53 season to check the previous season's results.

The season was unfavourable for tomato growing. During the trial period 22.79 in. of rain fell on 75 out of the 149 days, compared with a 97-year average of 15.01 in. for this period. In addition a heavy hail storm and a tropical cyclone caused considerable damage to many plants. These conditions favoured development of late-blight (*Phytophthora infestans* (Mont.) de Bary) infection which was particularly severe in check plots.

Experimental Methods

In September 1952, seedling tomato plants of the variety South Australian Dwarf were pricked out into boxes. During the period 1.10.52 to 10.10.52 they were infected by spraying with a broth culture of *C. michiganense*. The seedlings were planted in the field on 29.10.52. Counts of infected plants were made four days later. There were eight treatments replicated seven times. Replications were randomized and each consisted of a block of ten plants. Treatments were as follows:—

1. Bordeaux mixture: 2 lb. copper sulphate, 4 lb. hydrated lime in 100 gal. water.
2. Bordeaux mixture: 6 lb. copper sulphate, 8 lb. hydrated lime in 100 gal. water.
3. Thirospray (a proprietary mixture containing 50 per cent tetramethyl thiuram disulphide): 4 lb. in 100 gal. water.
4. Ascospray (a proprietary mixture containing 2½ per cent phenyl mercury chloride): 1 lb. in 100 gal. water.
5. Phygon XL (a proprietary mixture containing 50 per cent 2,3-dichloro-1,4-naphthoquinone): 1 lb. in 100 gal. water.
6. Check: sprayed with water.
7. Check: not sprayed.

The water-sprayed check was included to determine the effect of additional humidity such as is caused by the application of therapeutant sprays.

TABLE I. THE EFFECT OF VARIOUS THERAPEUTANTS ON TOMATO PLANTS INFECTED WITH BACTERIAL-CANKER AND LATE-BLIGHT

Treatment	Average weight of fruit per plant (lb.)	Average tomato weight (oz.)	Harvested fruit infected with Bacterial-canker (%)	Means of equivalent angles	Bacterial-canker infection on foliage and fruit	Harvested fruit infected with late-blight (%)
1. Bordeaux 2-4-100	4.50	2.37	63.6	52.91	Severe	1.6
2. Bordeaux 6-8-100	3.21	2.36	73.3	58.87	Severe	1.0
3. Thirospray 4 lb. per 100 gal.	9.44	2.69	31.2	33.93	Moderate	7.0
4. Ascospray 1 lb. per 100 gal.	6.76	2.82	30.0	33.21	Moderate	34.3
5. Phygon XL 1 lb. per 100 gal.	9.20	3.03	11.5	19.82	Slight	1.0
6. Check water-sprayed	3.61	2.74	37.4	37.67	Moderate	56.6
7. Check	3.06	2.52	35.0	36.29	Moderate	54.0
Difference necessary for significance 5%	1.49	0.35		3.95		
1%	2.01	0.46		5.30		

Sprays were applied at 7-day intervals from 4.11.52 until 23.3.53. Harvesting commenced on 19.1.53 and continued at 7-day intervals until 26.3.53. Records were taken of the total number and weight of fruit, and the number of fruits infected with canker in each block. Any fruit showing infection irrespective of number of lesions was recorded as "infected", no attempt being made to record severity of infection. Counts of harvested fruit infected with late-blight were made on 16.2.53, 23.2.53 and 10.3.53. In addition to the fruit counts, a survey of the trial was made on 23.2.53 to record severity of bacterial-canker infection on foliage and fruit.

Results

The count of infected plants prior to treatment showed that 98 per cent were infected with bacterial-canker. The infection was moderate (approx. 6 to 20 lesions per plant) at this time, and evenly distributed over the whole area.

The results of treatments are shown in Table I.

Discussion

The above results show that Phygon XL markedly reduced bacterial-canker infection, effectively controlled late-blight and had no apparent depressing effect on yield. There was no significant difference in canker infection between water-sprayed and unsprayed checks. Although they controlled late-blight, the weak and strong Bordeaux sprays both led to a considerable increase in bacterial-canker infection. Compared with Phygon XL and Thirospray, Bordeaux sprays caused a marked decrease in yield. From the data available it is not possible to indicate whether this decrease in yield was due to high canker infection or to possible spray damage. It was found in the Bordeaux treated plots that not only was a greater percentage of fruit infected with bacterial-canker but also that the infection on fruit and foliage was more severe than in any other treatment.

Thirospray gave good control of late-blight but did not significantly reduce canker infection and Ascospray was relatively ineffective against both late-blight and bacterial-canker.

Acknowledgment

Acknowledgment is due to Mr. W. B. Taylor, Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for statistical analysis of results.

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GRASS-GRUB POPULATIONS IN SOILS OF THE MANAWATU SAND COUNTRY

GRASS-GRUB POPULATIONS IN SOILS OF THE MANAWATU SAND COUNTRY

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Summary

The more extensive soils occurring in the Manawatu sand country are described briefly. Grass-grub populations found in the different soils during the spring of 1952 are indicated. It is shown that *Pyronota festiva* (Fab.) outnumbers *Costelytra* (*Odontria*) *zealandica* (White) in these soils.

Introduction

It has been obvious to workers concerned with the grass-grub problem in New Zealand that damage to pasture in some areas has been caused by larvae of the Scarabeid beetle *P. festiva*, the adults of which genus are commonly called "ti-tree beetles" because of their frequent association with *Leptospermum* spp. The present species, however, also thrives under conditions of open pasture land.

During 1950 and 1951 a search was made in the Manawatu district for areas of high grass-grub populations so that suitable sites could be selected for liberation of parasites arriving from Australia. During this work it was found that in the sandy soils, which are extensive here, grass-grub larvae of both *C. zealandica* and *P. festiva* were present, and that the latter species was of considerable importance.

In 1951 a soil survey was commenced in the district and offered a valuable opportunity to correlate soil type with grass-grub species. Descriptions of the soils and plant cover show ecological conditions under which the species exist.

The Soils of the Manawatu Sand Country

The Manawatu sand country consists of a complex of narrow dune ridges and intervening sandy flats. It covers an area of 80,000 acres in the Manawatu County and varies from eight to twelve miles in width. Except for small areas of peaty materials which have accumulated in depressions between dunes, or around lagoons, all the soils are formed from wind-blown sand.

Division of the soils into soil types, sub-types, and phases is based on differences in depth, colour, texture, structure, consistence, and drainage of the soil horizons. These features are an expression of the soil forming factors—parent material, climate, topography, vegetation, and the time these factors have been operating. Similar soils are grouped together into series and given a locality name, chosen from a district where they are typically developed.

The soil series mapped in the Manawatu sand country are the Waitarere and Foxton series on the dunes, and the Hokio, Himatangi,

Pukepuke, Carnarvon, and Omanuka series on the sand flats. Most of these soils have a coarse texture and their moisture-holding capacities are low. Offsetting this in many of them is a high water table which ensures a moisture supply sufficient to maintain high producing pastures.

All the soils were not sampled, but the range of soil conditions was covered by selecting one or more typical soils from the main series. No sampling of the Waitarere or Omanuka soils was done. The Waitarere soils consist of loose incoherent sand on which pastures have not been established. The Omanuka soils include peaty soils where surface water is present during the greater part of the year. No grass-grubs were present.

As these soils have not been described previously, brief descriptions of those sampled, grouped according to drainage, are given below. Maps showing the distribution of these soils are being prepared and will appear in a Soil Bureau bulletin.

A. *Poorly Drained Soils**

1. *Carnarvon Brown Loamy Sand*: Carnarvon brown loamy sand covers a small area west of Glen Oroua. It consists of 8 in. of very dark grey-brown loamy sand on a grey compact sand, containing many concretions of iron-cemented sand and some reddish mottlings.** The water-table is high, and surface water may be present for short periods during the winter and spring. Pastures consist mainly of ryegrass, crested dogstail, Yorkshire fog, meadow foxtail, and white clover.

2. *Carnarvon Black Sandy Loam*: Carnarvon black sandy loam is developed only north of Taikorea Road. It consists of 8 in. of a black sandy loam, overlying a grey sand containing many large concretions of iron-cemented sand and some reddish mottlings. The water-table is near the surface during the winter and spring. Pastures consist mainly of ryegrass, crested dogstail, Yorkshire fog, and white clover.

3. *Pukepuke Black Sand*: Pukepuke black sand occurs on the wetter flats in the Oroua Downs, Himatangi, and Foxton districts. The topsoil consists of 8 in. of very dark-brown to black sand with few dark red mottlings. This overlies a grey compact sand with many red and yellowish brown mottlings. With depth, the subsoil becomes blue-grey in colour and the mottling becomes less common. The water-table is high and surface water may be present for short periods during the winter and spring. Pastures consist mainly of ryegrass, crested dogstail, Yorkshire fog, and white clover.

4. *Hokio Sand*: Hokio sand is typical of the soils on the coastal flats. It consists of 1–3 in. of very dark brown sand on a compact bluish grey sand with faint yellowish brown and dark red mottling. Small shell fragments are common. The water table is comparatively

*Soil drainage classes according to definitions given in United States Department of Agriculture Soil Survey Manual.

**Grey and blue-grey colours indicate anaerobic reducing conditions in the soil. Where conditions are partly anaerobic and partly aerobic, reddish mottlings or concretions are present. Soils with these features are classified as gleyed soils. In strongly gleyed soils mottling is present in the topsoil, while in weakly gleyed soils it is deep in the subsoil. In these sandy soils gleying is due to a high water table. Non-gleyed soils show an even yellowish brown colour in the subsoil.

high and surface water may be present for short periods during the winter and spring. On low rises where the water table is low, the subsoil is looser and more strongly mottled. Pastures consist mainly of ryegrass, Yorkshire fog, and white and subterranean clovers.

B. *Moderately Well Drained Soils*

1. *Carnarvon Black Weakly Mottled Sandy Loam*: This soil is associated with the Carnarvon black sandy loam, but occurs on slightly higher ground where the water table is lower. The topsoil, consisting of 8 in. of a black sandy loam, overlies 4 in. of brownish yellow sand with few yellowish brown mottlings. This grades down into a yellowish grey sand, containing many concretions of iron-cemented sand. Pastures on this soil consist mainly of cocksfoot, ryegrass, and white clover.

2. *Himatangi Sand*: Himatangi sand is typically developed on the flats in the Himatangi district. The topsoil consists of 8–10 in. of black sand and this overlies a loose grey sand with few orange mottlings. Below 16 in. this grades into a grey sand with many red and yellowish brown mottlings. The water-table is low and pastures of ryegrass, cocksfoot, crested dogstail, and subterranean clover, dry off early in the summer.

C. *Excessively Drained Soils*

1. *Foxton Black Sand*: Foxton black sand is typical of the soils developed on the consolidated dunes within the sand country. It consists of 10–12 in. of a black sand, overlying a yellowish brown loose sand, which grades down at depth to a light brownish yellow sand. The topography of the dunes varies from easy rolling to steep. Because of the coarse texture of this soil, drainage is rapid and pastures dry off early in summer. These pastures consist mainly of browntop, cocksfoot, sweet vernal, *Microlaena,* ryegrass and subterranean and white clovers.

2. *Foxton Brown Sand*: Foxton brown sand is developed on the dunes in the eastern part of the sand country which formerly were forested. It consists of 8 in. of very dark brown sand overlying 6 in. of olive-yellow loose sand, which grades down into a greyish yellow sand. The dominant pasture plants are cocksfoot, ryegrass, crested dogstail, and subterranean and white clovers.

GRASS-GRUB POPULATIONS

Sampling Technique

In each soil, "sub-areas" approximately 500 yd. across were taken, and one-foot square samples were dug out at 50 pace intervals. The number of sub-areas in soils sampled ranged from 2 to 10, and in most cases the areas extended over 2 to 3 miles. Where sampling was carried out on dunes, the main aspects were sampled in approximately equal proportions. The depth to which the insects penetrated varied with the different soils and life-stages (larval or pupal), and soon became evident when sampling commenced. In locating the insects, drier soils were broken up with the hands, but wetter ones were sliced with a spade.

TABLE I. DETAILS OF GRASS-GRUB POPULATIONS IN SOILS

Soil and No. of Sub-areas.	*C. zealandica*					*P. festiva*				
	Total sq. ft. samples	Count range	Mean / sq. ft.	67% Confidence limits of mean	Life-stages*	Count range	Mean / sq. ft.	67% Confidence limits of mean	Life-stages	Date of sampling
Carnarvon brown loamy sand (4)	40	0	0	0	0	0	0	0	0	2.10.52
Carnarvon black sandy loam (4)	40	0–6	0.23	0.10–0.37	l.P.	0–4	0.15	0.00–0.34	L.	30.9.52
Pukepuke black sand (10)	100	0–8	0.41	0.32–0.51	L.P.	0–29	0.98	0.80–1.18	L.P.	17.9.52
Hokio sand (10)	100	0	0	0	0	0–60	7.69	6.89–8.57	L.p.	15.9.52
Carnarvon black weakly-mottled sandy loam (6)	60	0–8	0.50	0.37–0.64	L.P.	0–35	5.58	4.79–6.47	L.	30.9.52
Himatangi sand (10)	100	0–13	1.62	1.44–1.81	l.P.	0–30	6.23	5.56–6.96	L.P.	25.9.52
Foxton black sand (10)	100	0 21	4.30	3.94- 4.68	a.l.P.	0–33	4.64	4.12–5.21	L.P.	23.9.52
Foxton brown sand (2)	20	0–5	1.20	0.88–1.57	L.P.	0 7	1.05	0.65- 1.55	L.P.	7.10.52

* a = adult, l = larval, and p = pupal stage. Capitals indicate presence in large proportions.

RESULTS

Table I gives details of the incidence of each species in the different soils.

The more obvious features are the absence of *C. zealandica* in Hokio sand, although *P. festiva* is present in large numbers; the moderately large populations in Foxton black sand, Himatangi sand, Hokio sand, and Carnarvon black weakly-mottled sandy loam; the low populations in Foxton brown sand, Pukepuke black sand and Carnarvon black sandy loam; and the absence of grubs in Carnarvon brown loamy sand.

A statistical analysis of data for each species has been made and comparisons of the populations in the different soils are possible. The distributions are heavily skewed, and in order that comparisons of mean counts could be made the transform log $(1 + x)$ was used since mean and standard deviation are approximately proportional. The procedure of transformation of the data allows confidence limits to be computed for means and these are shown in Table I. Results are as follows:

A. *C. zealandica*

1. The mean count for Foxton black sand is significantly greater than that for any other soil (at the 1 per cent level).

2. The mean counts for Foxton brown sand and Himatangi sand do not differ significantly, but are significantly greater than those for any other of the remaining soils (at the 5 per cent level).

3. None of the mean counts for Carnarvon black weakly-mottled sandy loam, Carnarvon black sandy loam, Carnarvon brown loamy sand, Hokio sand or Pukepuke black sand differ significantly from each other.

B. *P. festiva*

1. The mean counts for the four soils Hokio sand, Himatangi sand, Carnarvon black weakly-mottled sandy loam, and Foxton black sand, are all significantly greater than those for any of the remaining soils (at the 5 per cent level).

2. Within these four soils the mean for Hokio sand is significantly greater than either of the means for Carnarvon black weakly-mottled sandy loam, or Foxton black sand (at the 5 per cent level); Himatangi sand takes an intermediate position not differing significantly from the others.

3. The means of the three remaining soils in which *P. festiva* is present do not differ significantly.

DISCUSSION

This account indicates that considerable grass-grub populations occur in the Manawatu sand country soils, and that *P. festiva* is the

most numerous species present. Spring sampling does not indicate maximum grass-grub populations, however, because there is certain to be winter mortality; but most grubs are in their final instar in spring, at which stage the differentiation of the species presents no difficulties.

Although *P. festiva* is the smaller species, its high populations and attendant damage in the absence of *C. zealandica* indicate its importance. The factors responsible for clumping of populations (skewed distribution) are not known, but it may be related to initial oviposition, to congregation because of larval feeding preferences, to local variation in terrain, or to the effects of parasites, e.g., larvae of Asilid flies.

The low numbers of grubs in most of the poorly drained soils suggests that the presence of a high water-table may be a factor limiting grass-grub populations in the sand country soils. In Hokio sand, a poorly drained soil, the number of grubs is high, but observations made during the sampling indicated that the grubs are concentrated in the drier rises, and are almost absent from the low-lying wetter parts.

The factors responsible for the absence of *C. zealandica* from Hokio sand are likely to be complex.

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CORRIGENDA

NEW ZEALAND JOURNAL OF SCIENCE AND TECHNOLOGY A35: 117-26

Measurement of feed-intake by grazing cattle and sheep. III.

p. 122. line 17. Formula should read $E = \frac{g}{c}$

NEW ZEALAND JOURNAL OF SCIENCE AND TECHNOLOGY A35: 327-59

Effects of sheep dung and urine on a soil under pasture at Lincoln, Canterbury, with particular reference to potassium and nitrogen equilibria.

p. 343. Table IX. In box-heading, "Total K" should read: "Total K_2O."

p. 358. Journal reference of paper by Elgabaly, M. M. and Wiklander, L. should be amended to read: *Soil Sci. 67*: 419-24.

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